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# THE MOUNT SINAI JOURNAL OF MEDICINE

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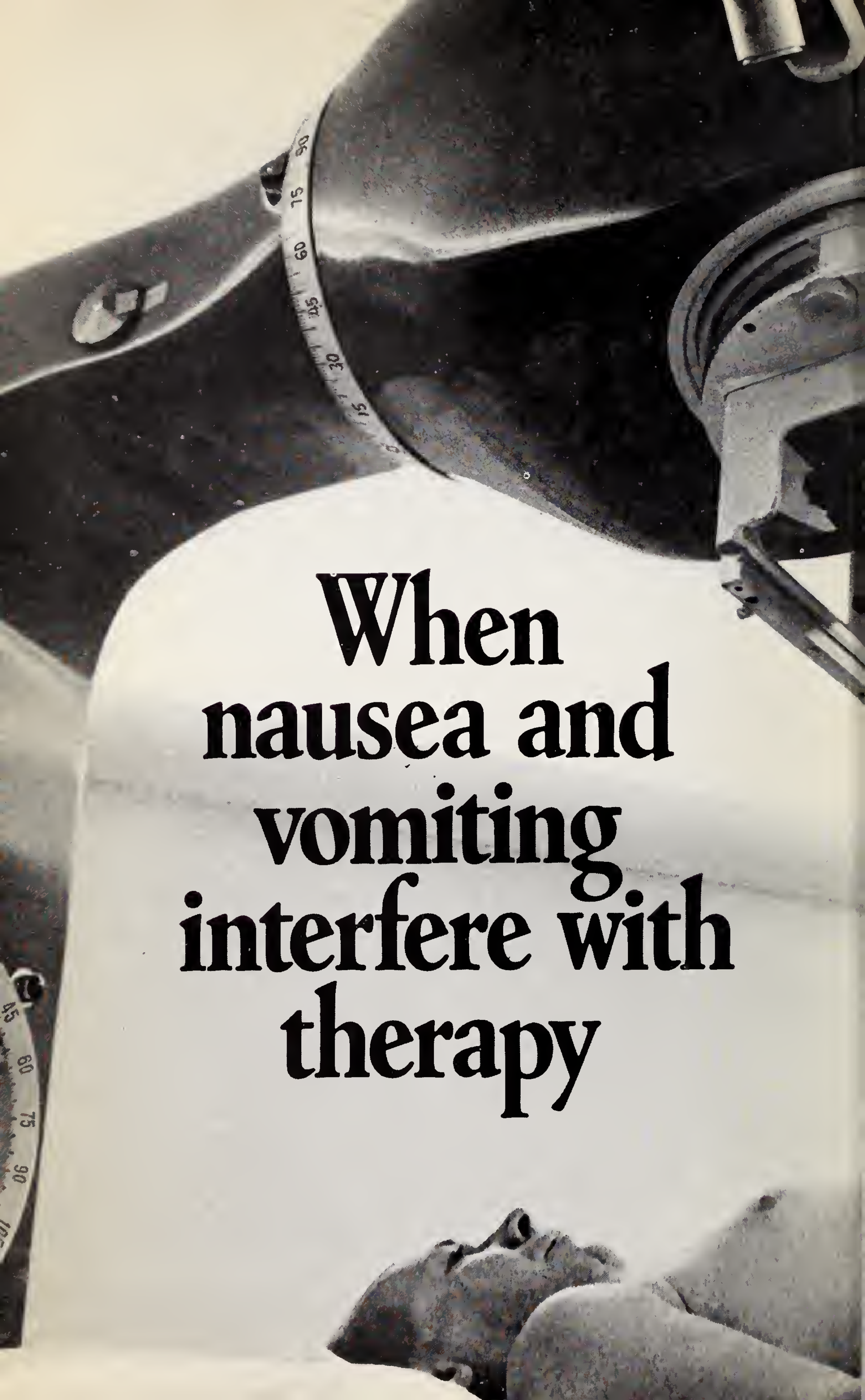
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# Small-Cell Tubular Carcinoma of the Breast—Observations on the Role of Local Excision in the Treatment of This Particular Cancer

CYRIL TOKER, M.D.

## Abstract

Experience with twelve breast cancers, removed from eleven patients by local excision, is presented. No other form of therapy was employed. Five of the tumors were tubular carcinomas composed of small- to medium-sized cells. There have been no recurrences in these patients, over follow-up periods that range from six to 14 years. Seven tumors were trabecular in structure. Their cell size was small to medium. Two patients have developed recurrent carcinoma, with one fatality. The remaining five are free of recurrence over periods that vary from seven to 20 years. It is believed that some basis exists for the further evaluation of local excision as a definitive form of therapy in cases of tubular carcinoma of the breast. It is emphasised, however, that this recommendation applies solely to the tubular carcinoma composed of small- to medium-sized cells. Lesions containing large cells, or composed mainly of aglandular cords (trabeculae) are excluded, even though some tubular differentiation be present in areas. Until further information has been accumulated, it may be prudent to restrict this conservative approach to small tumors.

Small-cell tubular carcinomas constitute an uncommon but distinct category of breast cancers (1). Orderly, well-developed glands, composed of cells that lack the cytologic stigmata of malignancy, suggest an indolent clinical behavior, and these tumors are considered to be less aggressive than the more commonly encountered forms of mammary carcinoma (1).

A current trend toward less radical surgery for breast cancer is apparent (2, 3). In view of this trend it is appropriate to present a small series of breast cancers that have been treated by local excision alone. The tumors under consideration presented a uniform cytology. Two groups were, however, discernible on the basis of cellular organization—a glandular or tubular group, and an aglandular or trabecular group.

## Material and Observations

Twelve tumors removed by local excision from the breasts of eleven women were studied. No other therapy was employed. Follow-up information is available for periods that range from three to 20 years. The follow-up is continuing. Table I provides a summation of the results to date.

Five tumors were composed mainly of glandular structures (Cases 1–5). Representative areas are illustrated in Figures 1 to 3. Cells were classified according to nuclear size, for two reasons. Nuclear, rather than cytoplasmic, size and structure relate more directly to neoplastic activity. Cytoplasmic boundaries

From the Department of Pathology, Mount Sinai School of Medicine of The City University of New York, N.Y. 10029.

TABLE I

| Case | Age at Surgery | Size of Tumor | Morphology of Tumor | Period of Follow-Up | Recurrence |
|---|---|---|---|---|---|
| 1 | 50 | 0.9 cm | Tubular, medium & small cells | 14 years | None |
| 2 | 49 | 1.2 cm | Tubular, small & medium cells | 11 years | None |
| 3 | 50 | 0.9 cm | Tubular, small cells | 9 years | None |
| 4 | 50 | 0.4 cm | Tubular, small & medium cells | 7 years | None |
| 5 | 46 | 0.8 cm (L) | Tubular, small & medium cells<br>Trabeculae present | 6 years | None |
|  |  | 0.8 cm (R) | Trabecular with some tubules<br>Small & medium cells | 6 years | Recurrent tumor after 5 years. Mastectomy. |
| 6 | 32 | 0.5 cm | Trabecular, medium cells | 4 years | Recurrence after 5 months. Mastectomy. Death 4 years after local excision |
| 7 | 45 | Intraductal carcinoma with microscopic focus of invasion | Trabecular, medium cells | 7 years | None |
| 8 | 46 | 0.2 cm | Trabecular, small cells | 7 years | None |
| 9 | 43 | 0.7 cm | Trabecular, medium cells | 13 years | None |
| 10 | 72 | 0.8 cm | Trabecular, small & medium cells | 19 years | None |
| 11 | 45 | 1.2 cm | Trabecular, small & medium cells | 20 years | None |

are, furthermore, frequently obscure or inapparent. Nuclear membranes are usually distinct. Small nuclei approximated the size of red blood corpuscles, 7 microns. Medium-sized nuclei measured some 14 microns in diameter. Nuclei considered to be large (21 microns or more) were not encountered in this study. The five tubular carcinomas exhibited varying admixtures of small- to medium-sized cells. Mitotic activity was not conspicuous. Nucleoli were visible within medium-sized nuclei, but were not large. Tumor sizes varied between 0.4 and 1.2 cm. Follow up periods range from six to 14 years. There have been no recurrences to date.

The remaining seven tumors—1 patient had bilateral infiltrating carcinomas—manifested cytologic characteristics similar to those of the first group. These have been detailed above. Small- and medium-sized cells were seen. Large cells were not encountered. The organization of the cells differed, however, from that described in the first category. Solid, aglandular cords (or trabeculae) either predominated or comprised the entire substance of these growths. Tubules were present in some instances, but the trabecular element formed the greater part

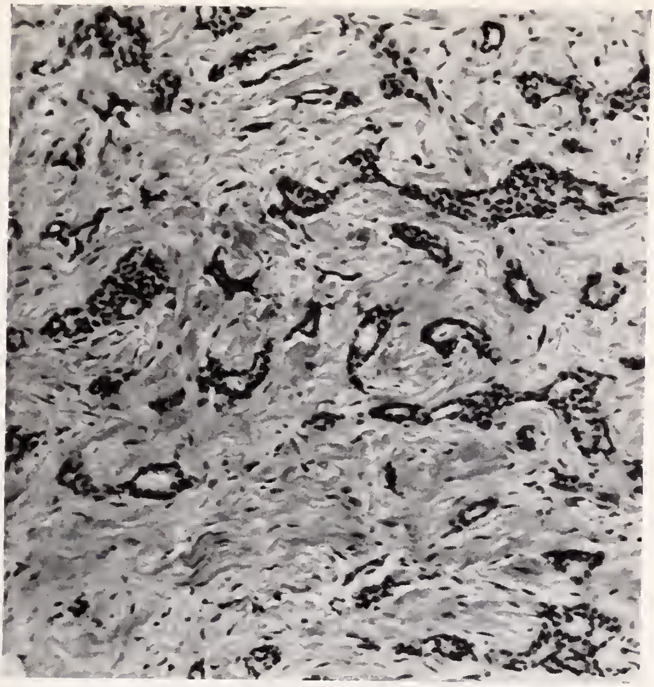

Fig. 1. Case 5. Left breast. Tubules composed of small cells are shown. H&E × 80.

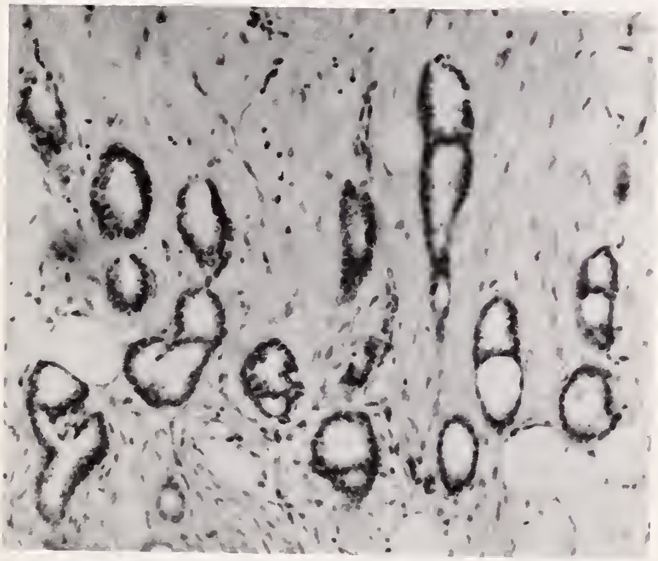

Fig. 2. Case 2. Regular tubules composed of small- to medium-sized cells, are shown. H&E × 80.

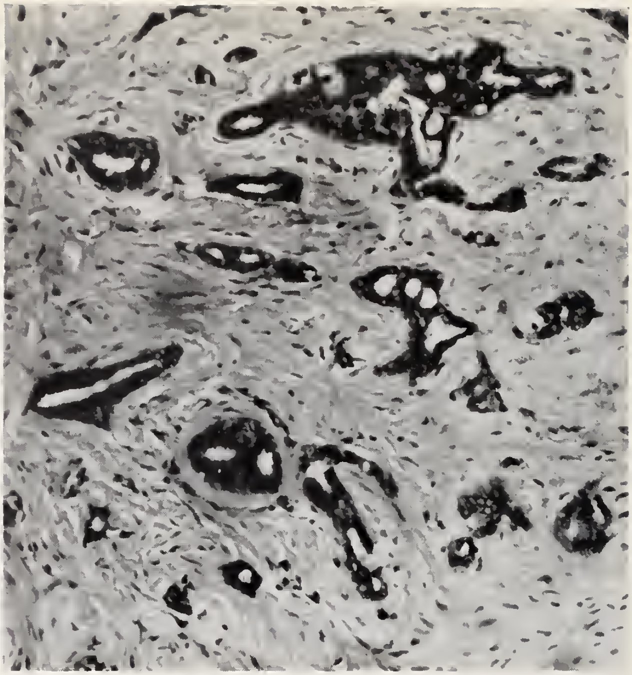

FIG. 3. Case 4. Tubules composed of medium-sized cells are shown. H&E × 80.

of the growth. Trabecular patterns are illustrated in Figures 4 and 5. These trabecular carcinomas ranged in size from microscopic dimensions to 1.2 cm.

Follow-up information is available for periods up to 20 years. Two tumors have recurred. In the first instance there was recurrence after a period of five months. Mastectomy was performed, but the patient expired of disseminated mammary carcinoma some four years subsequent to the initial local excision. In the second instance recurrence supervened some five years after local excision. The resected breast exhibited three independent foci of infiltrating carcinoma. Both tubular and trabecular patterns were present within the recurrent tumors, which retained the small- to medium-sized cells observed within the initial lesion. Large cells were not encountered. A year has elapsed subsequent to mastectomy, and follow-up is continuing. The remaining five tumors have not recurred.

## Comment

Five tubular carcinomas treated solely by local excision have not recurred over follow-up periods that range from 6 to 14 years. These tumors consisted of small- to medium-sized cells. Two out of 7 trabecular carcinomas similarly treated have recurred, with 1 fatality. The trabecular lesions, like the tubular, were composed of small- to medium-sized cells.

With regard to the trabecular carcinomas, neither an innocuous cytology nor a

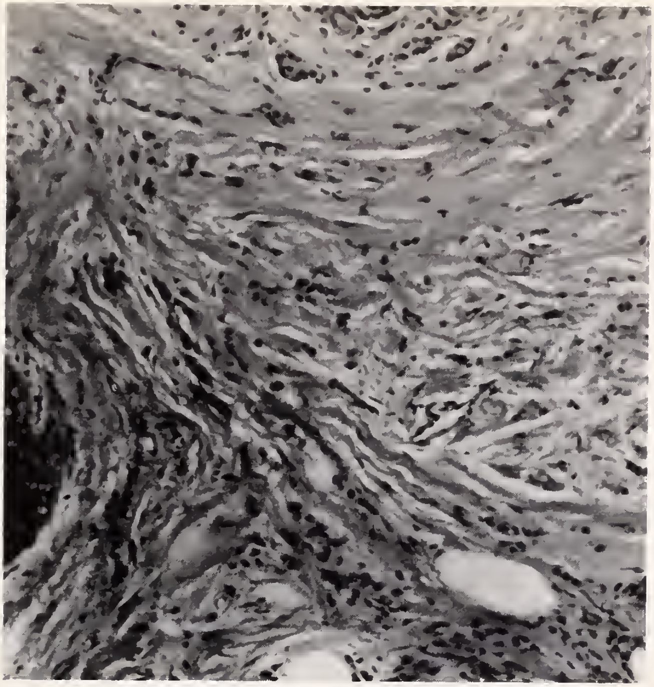

FIG. 4. Case 8. Small cells form slender cords. H&E × 80.

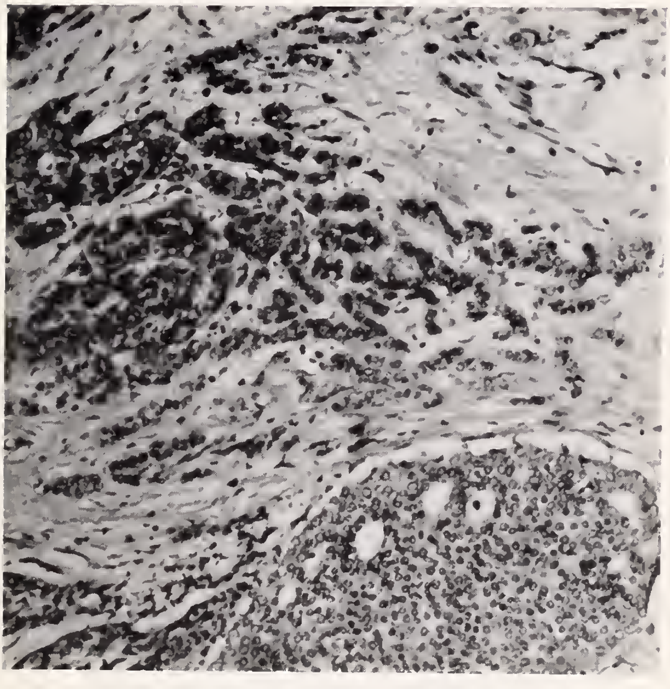

FIG. 5. Case 7. Intraductal carcinoma is seen in the lower part of the figure. Above the ducts infiltrating trabeculae are seen. These are composed of medium-sized cells. H&E × 80.

diminutive size preclude devastating recurrence after local excision. Our present concern is with the tubular carcinomas.

Both the glandular differentiation and the growth pattern of the tubular carcinomas suggest a low order of malignancy. The tumor may be so well differentiated as to be mistaken for benign sclerosing adenosis. In view of these considerations the necessity for mastectomy in the treatment of this specific form of mammary carcinoma may be questioned. Although the number of cases presented is small, it is believed that the results provide some basis for the further investigation of local removal as a definitive form of therapy in these cases, particularly where the lesion is small. The author hesitates to make a similar recommendation in the case of a large tumor, until further information is available with regard to the smaller lesions.

It is to be emphasised that the presence of tubular structures does not, per se, denote a low grade of malignancy. It is essential that the cytology of the lesion be carefully evaluated. Anaplastic, large-cell carcinomas may manifest focal glandular differentiation. The recommendation is made solely with reference to tubular carcinomas composed, preferably, of small cells. Some admixture of medium-sized cells may be acceptable. However, the presence of large cells would, at this time, prevent this author from recommending local removal. The presence, similarly, of a significant or dominant trabecular component would militate against a recommendation for conservative surgery.

In summary, the suggestion that local removal be practiced applies solely to the small- to medium-cell tumor that is composed mainly of tubular structures. This evaluation cannot be made from the frozen section. Multiple paraffin sections are required in order to detect the presence of larger cells or trabeculae. A final determination should, therefore, not be made at the time of the biopsy.

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# The Hyperbaric Chamber and Pulmonary Oxygen Toxicity: A Clinical and Pathologic Study

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Mechanical devices to maintain oxygenation are in common use. However, the hyperbaric chamber and membrane oxygenator are limited to a few centers. This communication is of special interest because it reports on a relatively large experience with the hyperbaric chamber in patients with acute respiratory failure. Furthermore, the authors have made careful observations of the clinical and pathologic changes encountered in oxygen toxicity. As a bonus, a patient treated with hyperbaroxia plus the membrane lung is reported and a future role for this latter apparatus is proposed. *Editor's note*

Because of the availability of the hyperbaric chamber at The Mount Sinai Hospital, many patients dying of respiratory failure after good conventional therapy had failed have been referred to the Hospital. It was hoped that increasing the partial pressure of oxygen would supply adequate oxygen to sustain life. Only a few of these patients were salvaged and the others died, either because there was insufficient functioning lung tissue to support life or, more often, because oxygen toxicity was superimposed on the original pulmonary pathology by hyperbaroxia. This paper is a retrospective study of all the patients treated in the hyperbaric chamber for respiratory failure from the first patient seen in 1965 until December 31, 1971.

## Material and Methods

Thirty patients in acute respiratory failure were admitted to the hyperbaric chamber at The Mount Sinai Hospital during the period from 1965 to 1971. They ranged in age from two years to 70 years. Eleven were female and nineteen were male. Of the 30 patients, eight survived.

Patients were rated, using the code in Table I, according to the amount of oxygen therapy received. Records were obtained from referring hospitals when patients were transferred; and referring physicians were questioned concerning details of oxygen therapy. Arterial blood gases were analyzed by the electrode method and inspiratory oxygen concentration ($F_{IO_2}$) was measured with a Beckman paramagnetic oxygen analyzer (Beckman Model D2).

In the 22 patients who died, 13 postmortem examinations were obtained; and in the eight survivors, one lung biopsy was performed.

Pathologic examinations were performed on representative sections of lung stained with routine hematoxylin eosin and connective tissue stains. On the basis of histologic features, the specimens were divided into three groups. The first group (Fig. 1) showed only exudative lesions primarily restricted to the alveolar lining cells. These included hyperplasia, metaplasia, focal necrosis or complete loss of epithelium, elevation hypertrophy of type 2 alveolar lining

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TABLE I

*Code for Therapy*

| Oxygen | Time | Method | |
|---|---|---|---|
| 20–40% 1+ | 24 hours A | N.C. | nasal catheter |
| 40–60% 2+ | 24–48 hrs. B | F.M. | face mask |
| 60–80% 3+ | 48–72 hrs. C | O.T. | orotracheal tube |
| 80–100% 4+ | 72+ hours D | N.T. | nasotracheal tube |
| | | T.T. | tracheostomy tube |
| | | HBC | hyperbaric chamber |
| | | MO | membrane oxygenator |

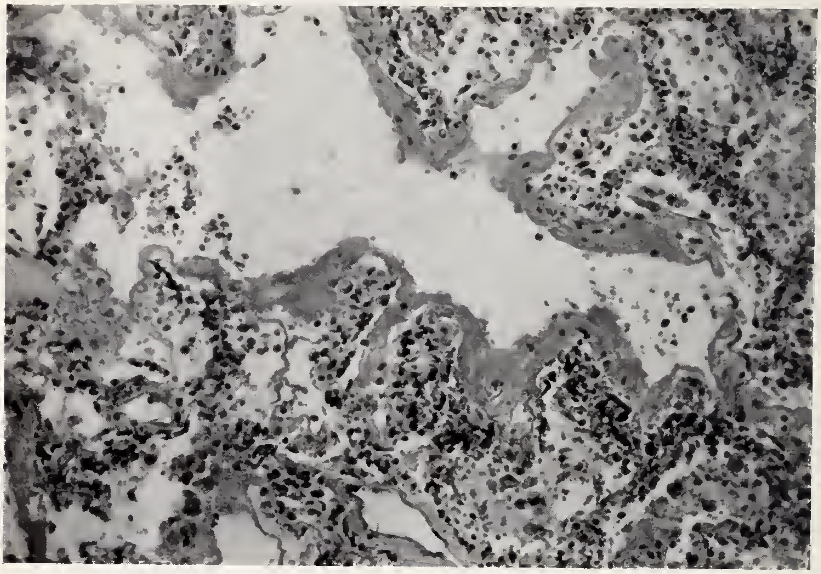

Fig. 1. Exudative phase. The alveoli are lined by hyaline membranes and the lumina are filled with desquamated, necrotic epithelium. Marked hyperplasia of Type 1 and Type 2 epithelial cells and interstitial edema are also present.

cells and necrosis of type 1 cells, and edema, hemorrhage and hyaline membrane formation in the lumen. A strict criterion in this group was the absence of increased collagen tissue and interstitial fibroblasts. The second group classified as intermediate showed both exudative lesions and focal proliferative changes. The third group (Fig. 2), the proliferative phase, showed marked fibrosis with the appearance of fibroblasts in the interstitium, replacement of the interstitial cellular elements by loose edematous connective tissue, and obliteration of the alveolar spaces. Changes in bronchial epithelium were observed in each of the three groups.

Patients' records were reviewed to determine whether corticosteroids or other medication which might influence tissue reaction had been administered. Blood coagulation studies were performed. The kidneys, liver, spleen and heart, when available, were studied for pathologic changes.

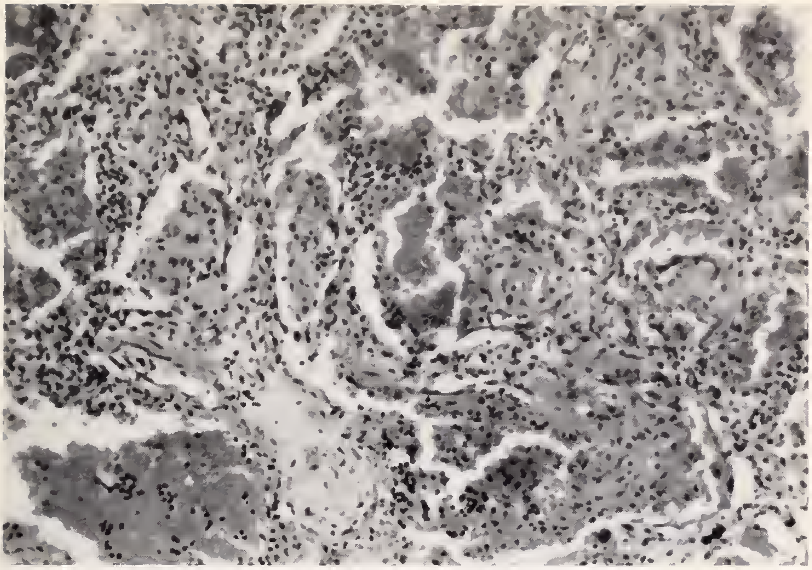

FIG. 2. Proliferative phase. Diffuse interstitial fibrosis (collagenic and mesenchymal cells) is noted. The alveoli are filled with hemorrhage and are lined by remnants of flattened type 2 epithelial cells.

## Results

The results of these studies are summarized in Table II. There were 11 cases classified as viral pneumonia, in six of which Hong Kong A2/68 virus was identified at some time in the course of the disease. All of the latter six cases occurred between December, 1968 and March 1969. There were six aspiration and four bacterial pneumonias. Very often bacteriologic studies were negative, perhaps due to prolonged antibiotic therapy at other hospitals.

Of the 30 patients, 19 had been treated at other hospitals and were sent to us in terminal respiratory distress. Twenty-one cases received 100 percent oxygen for more than 24 hours at 1 ATA (one atmosphere absolute) and most of these received additional days of 100% oxygen with periods of pressure to 2 or 3 ATA.

Of the 13 postmortem examinations performed, seven patients were classified by the pathologist as in the exudative group. All of these patients had received corticosteroids. Three patients were classified as intermediate, two of whom received corticosteroids. Three cases were classified as proliferative and none of these had received corticosteroids.

It can be seen from Table III that radiologic diagnosis is of little help in distinguishing the type of pulmonary lesion. However, in many cases (Figs. 3a–3c), clearing of the initial disease process and the appearance of a new lesion suggests the development of pulmonary oxygen toxicity. In none of these patients was oxygen the initial cause of pulmonary disease since all of the patients were already in some respiratory distress before oxygen therapy was given.

TABLE II

*Summary of Results*

| Case No. | Age | Sex | Clinical Diagnosis | Radiologic Diagnosis | Oxygen Therapy | Steroids | Out-come | Pulmonary Pathology |
|---|---|---|---|---|---|---|---|---|
| 1 | 16 | M | Viral pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ BTT | Yes | Well | None |
| 2 | 45 | M | Goodpasture's syndrome | Bilat. diffuse pulm. infiltr. | HBC 4+ AOT | Yes | Died | None |
| 3 | 2 | M | Viral pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ CTT | Yes | Died | Exudative phase with hyaline membranes |
| 4 | 62 | M | Aspiration pneumonia | Rt. lung diffuse infiltr. (had previous L. pneumonectomy) | 4+ BOT | Yes | Died | None |
| 5 | 32 | F | Aspiration pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ ATT | Yes | Well | None |
| 6 | 40 | M | Viral pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4 + ATT | No | Well | None |
| 7 | 14 | F | Bacterial pneumonia | Complete consolid. R. lung; partial consolid. L. lung | HBC 4+ ATT | Yes | Well | None |
| 8 | 70 | M | Viral pneumonia | Normal | 1 +ANC | No | Well | None |
| 9 | 50 | F | Aspiration pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+DTT | Yes | Died | Exudative phase with hyaline membranes |
| 10 | 40 | M | Pneumocystis carinii ( prolonged steroid therapy) | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | Yes & methotrexate | Died | Intermediate phase with hyaline membranes & erosion of trachea |
| 11 | 49 | M | Aspiration pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | Yes | Died | None |
| 12 | 51 | M | Idiopathic pulm. fibrosis w. acute infection | Bilat. diffuse pulm. infiltr. | 1 + DFM | Yes + INH | Well | None |
| 13 | 59 | F | Chr. obstr. lung disease + viral pneumonia | Bilat. diffuse pulm. infiltr. | 4 + CTT | Yes | Died | Exudative phase with thickened alveolar walls |
| 14 | 58 | M | Viral pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | No | Died | Proliferative phase |
| 15 | 56 | M | Viral pneumonia chronic lymphatic leukemia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | Yes + leukeran | Died | Exudative phase with hyaline membranes |
| 16 | 33 | F | Viral pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | Yes | Died | Exudative phase with hyaline membranes |
| 17 | 61 | M | Viral pneumonia | Bilat. diffuse pulm. infiltr. | 4 + AOT | No | Died | None |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 18 | 42 | F | Viral pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | No | Died | Intermediate phase with hyaline membranes |
| 19 | 25 | M | Pulm. fat embolus | Bilat. diffuse pulm. infiltr. | HBC 4+ ATT | No | Died | None |
| 20 | 30 | F | Bilateral bronchopneumonia | Bilat. diffuse pulm. infiltr.; fluid L. base | HBC 4+ DTT | Yes | Died | Exudative phase with hyaline membranes |
| 21 | 21 | M | Bilateral bacterial pneumonia | Bilat. diffuse pulm. infiltr. | 4 + DTT | Yes | Died | Exudative phase with hyaline membranes |
| 22 | 27 | F | Aspiration pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | Yes | Died | None |
| 23 | 31 | M | LLL bronchopneumonia | Complete consol. LLL | HBC 4+ DTT | Yes Amphotericin B | Died | None |
| 24 | 33 | F | Aspiration pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | Yes | Died | None |
| 25 | 51 | M | Viral pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT | Yes + amphotericin B + INH | Died | Intermediate phase with hyaline membranes |
| 26 | 38 | M | Chemical pneumonitis p anesthesia | Bilat. diffuse pulm. infiltr. | 2 + DTT | No | Well | None |
| 27 | 27 | F | Desquamative interstitial pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4 + ATT and 2 +D | Yes | Well | Desquamative interstitial pneumonia |
| 28 | 56 | F | Pulm. edema, cong. heart failure | Bilat. diffuse pulm. infiltr. | HBC 4 + ATT | No | Died | None |
| 29 | 11 | M | Fecal peritonitis gram. neg. sepsis & shock; myocarditis, pulm. edema | Bilat. diffuse pulm. infiltr. | 4 + DTT | No | Died | Proliferative phase |
| 30 | 20 | M | Aspiration pneumonia | Bilat. diffuse pulm. infiltr. | HBC 4+ DTT M.O. | No | Died | Proliferative phase |

TABLE III

*The Effect of Corticosteroids*

| Pt. No. | Corticosteroids | Treatment Code | Pathology Dx |
|---|---|---|---|
| 3 | Yes | HBC 4+C TT | Exudative |
| 9 | Yes | HBC 4+D TT | Exudative |
| 13 | Yes | 4 + C TT | Exudative |
| 15 | Yes and Leukeran® | HBC 4+D TT | Exudative |
| 16 | Yes | HBC 4+D TT | Exudative |
| 20 | Yes | HBC 4+D TT | Exudative |
| 21 | Yes | 4 + D TT | Exudative |
| 10 | Yes + Methotrexate® | HBC 4+ D TT | Intermediate |
| 18 | No | HBC 4+ D TT | Intermediate |
| 25 | Yes + amphotericin B® | HBC 4+ D TT | Intermediate |
| 14 | No | HBC 4+ D TT | Proliferative |
| 29 | No | 4 + D TT | Proliferative |
| 30 | No | HBC 4+ D TT MO | Proliferative |

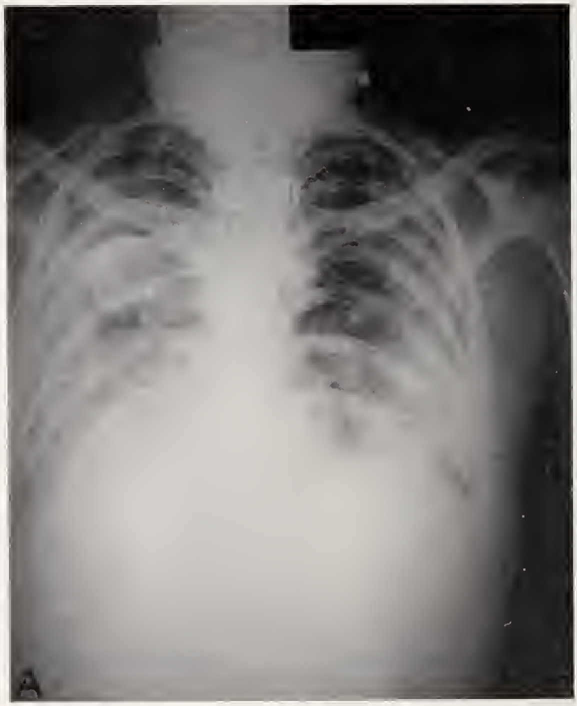

FIG. 3a.

FIG. 3. Patient no. 24. a. 24 hours after admission; clinical diagnosis: aspiration pneumonia. Diffuse bilateral patchy infiltrates noted. b. 48 hours after admission, clinical condition improving, clearing of initial disease process. c. Eight days after admission, in terminal respiratory failure, extensive bilateral pulmonary densities involving both lungs.

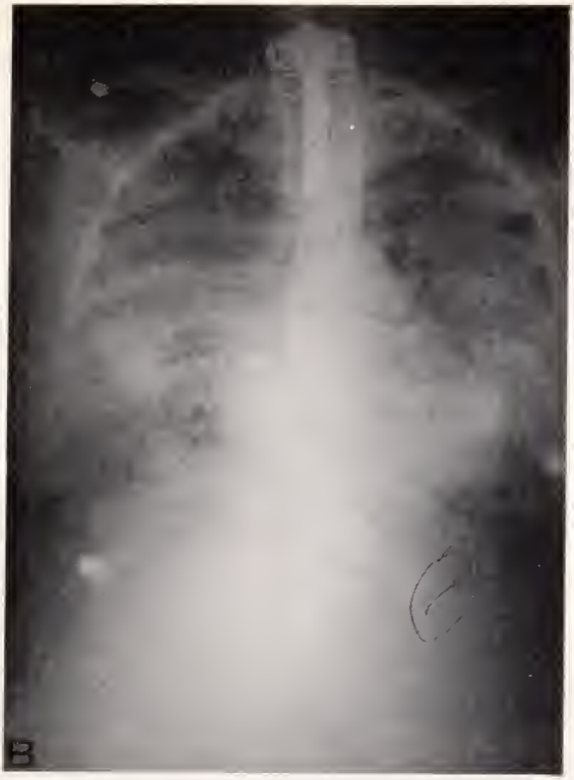

FIG. 3b.

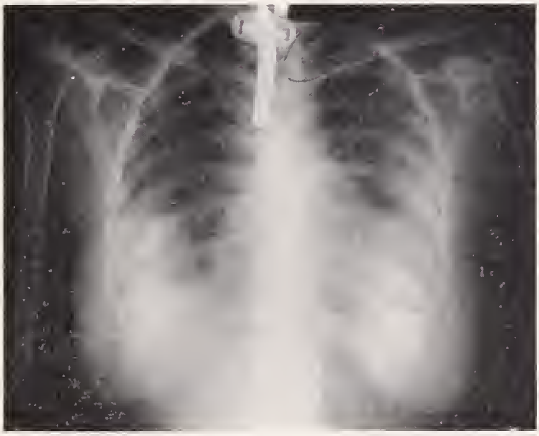

FIG. 3c.

## Discussion

Since the advent of mechanical ventilators and the use of an endotracheal tube or tracheostomy to treat respiratory failure, it has been possible to deliver high concentrations of oxygen for long periods of time to patients who in the past would have succumbed early in their illness as a result of hypoxia. If these patients do not recover sufficiently in a few days to allow the concentration of inspired oxygen to be reduced to 60 percent or less, a new hazard appears, pulmonary oxygen toxicity.

In general, in retrospective studies of patients who died after prolonged mechanical ventilation and exposure to high concentrations of inspired oxygen, the investigators were unable to show a cause and effect relation between oxygen and the lung lesions. Two recent prospective studies (1, 2) from the same department are particularly perplexing. One study of patients following open heart surgery suggested that ventilation with 100 percent oxygen for up to 48 hours produced no detectable changes in pulmonary function when compared with a similar group of patients ventilated with 42 percent or less oxygen. The other study of patients with irreversible brain damage compared patients ventilated with air to those receiving 100 percent oxygen. The group receiving 100 percent oxygen demonstrated significantly greater impairment of lung function. Obviously, these disparate conclusions cannot be reconciled.

Our study is unique in that 22 of 30 patients were treated in the hyperbaric chamber at increased ambient pressures. In addition, the following significant factors were noteworthy.

1. The respiratory disease was extremely severe. Many patients were sent from other medical centers because they could no longer be treated by conventional care.
2. Two of the authors were involved in the clinical care of all patients and kept detailed records of the respiratory therapy.
3. Steroids were used in two-thirds of the patients.
4. Radiographic studies in many patients showed clearing of the initial pathology and then development of a new process.
5. Correlation of the pulmonary pathology at postmortem with the duration and amount of oxygen therapy was possible.

As expected, the hyaline membrane which appears to be the early manifestation of alveolar lining cell damage was rare in the more advanced cases. The incidence of bronchopneumonia and interstitial round cell infiltration was more prevalent in the early phase; however, the exact estimation of the extent of the bronchopneumonic process was impossible. All fourteen patients from whom pathologic tissue was examined had received large amounts of oxygen. Seven patients whose lungs were found to be in the exudative stage also received corticosteroids and one was Leukeran.® Despite the large amounts of oxygen there were no signs of the late proliferative pathologic changes in these seven. Two patients whose lungs were classified as in the intermediate phase received corticosteroids and one of these had received methotrexate® for treatment of a chronic skin disease. Of 4 patients who received no corticosteroids, one was classified as in the intermediate and 3 as in the proliferative phase (Table IV). This might suggest that corticosteroids help to slow the progressive idiopathic fibrosing process and prevent the development of later and probably irreversible fibrosis caused by prolonged oxygen therapy. However there were too many other variables operative in these four patients to support this contention.

In our series of patients almost all were treated with ethacrynic acid or furosemide at some time in their course. In no case did the diuretic agent improve the status of the lungs. Heparin, which reduces intravascular coagulation, platelet aggregation, serotonin release and decreases the likelihood of clot propagation, was given to 2 patients with no discernible effect: the patient who had thrombocytopenia and the patient who had fat emboli.

The diagnosis of pulmonary oxygen toxicity is extremely difficult and can only be presumed when the postmortem findings in the lungs correlate with known prolonged time and high concentrations of oxygen administration. As our clinical experience with these patients grew, we reduced the $\mathrm{FIO}_2$ and accepted arterial blood oxygen levels which would have been unacceptably low to us in previous years. When multiple manipulations of antibiotic therapy produced no improvement whereas reducing the $\mathrm{FIO}_2$ did, we assumed that oxygen toxicity had supervened. In some cases, during the period of high oxygen exposure, radiographic lung lesions attributed to infection showed evidence of healing whereas diffuse alveolar infiltrates characteristic of oxygen toxicity progressed. This was noted in six of our patients. The radiologic lesions of oxygen toxicity appeared as diffuse bilateral pulmonary densities which were irregular in shape. Continued exposure to oxygen was accompanied by extension and coalescence of infiltrates until, in the most severe cases, almost the entire lung was densely opacified. In some, the appearance initially resembled pulmonary edema or hyaline membrane disease of premature infants. Nine patients had pneumothoraces due to the increasing inflation pressures needed in the face of decreasing compliance.

The major difficulty in making a definitive diagnosis of pulmonary oxygen toxicity, especially in the early stages, is the relatively nonspecific nature of the radiologic changes, particularly in a patient in whom there are likely to be several simultaneous causes for the development of diffuse bilateral pulmonary densities. These include early pulmonary edema, aspiration pneumonitis, viral pneumonia, alveolar proteinosis and extensive pulmonary embolism due to blood clot or fat. In all of our cases, at least one of these conditions existed prior to the administration of oxygen therapy and produced the respiratory failure that necessitated prolonged and intensive oxygen therapy. Correlation between clinical and laboratory findings and accurate knowledge of the inspired oxygen content and length of time of administration are necessary to make a presumptive diagnosis, and pathologic proof is generally required for its confirmation.

It is hazardous to conclude from isolated experiences that oxygen toxicity is reversible, especially since it is difficult to determine whether transient pulmonary densities represent evidence of clearing pulmonary oxygen toxicity or another acute inflammatory process. We have encountered two patients in the series who we believe survived because we saw them late in our experience and we had learned enough to stop the high oxygen concentration and administer the lowest $F_{IO_2}$ compatible with survival. This minimal acceptable $P_{O_2}$ varies in each case and is not necessarily reflected by the $Pa_{O_2}$ since the two survivors lived for intervals of 10–12 hours with a $Pa_{O_2}$ of 35–50 mmHg. Level of consciousness, adequacy of circulation and patient comfort are often vital indicators of adequacy of oxygenation. Hyde (3) has shown that when the inspired $P_{O_2}$ was lowered below 0.85 atm clearing of pulmonary infiltrates could be detected in five to nine days and was complete in 23 to 46 days. The time of onset of radiological changes due to oxygen toxicity varied with inspired $P_{O_2}$ and exposure conditions. In one group of patients (4), abnormalities were not detected before at least 60 to 70 hours of continuous administration of 90 to 100 percent oxygen at 1 ATA. In another series (5), roentogenographic changes were found after only 20 hours of pure oxygen breathing. These discrepancies probably can be accounted for by preexistent lung disease, patient variation, drug therapy, and methods of mechanical ventilation. In our patients about three days of 80 to 100 percent oxygen at 1 ATA were necessary before roentgenographic changes attributable to oxygen were observed.

## Management

In the management of our series of patients it was hoped that intermittent exposure to high concentrations of oxygen at increased ambient pressures and then a return to a normal level would increase pulmonary oxygen tolerance. This is consistent with the concept proposed by Haugaard (6) that oxygen poisoning results from oxidation of critical enzymes or cofactors which can be readily resynthesized when a nontoxic $P_{O_2}$ is restored. In our patients the increase in oxygen tolerance achieved by periodic interruptions of oxygen breathing appeared to depend at least partially upon the degree of oxygen intoxication which developed before reduction of the $F_{IO_2}$. Five of 21 patients were salvaged by intermittent hyperbaroxia who probably would have died if left to conventional treatment alone.

Our patients were sedated to the point where they were asleep but could be aroused by direct stimulation. This was achieved by frequent intravenous doses of morphine sulfate. In severe cases curare or succinylcholine hydrochloride were used to paralyze the patient temporarily and thereby achieve controlled ventilation. Constant attendance is absolutely necessary since accidental interruption of the respirator is fatal.

Positive end-expiratory pressure (PEEP) is often useful when compliance is poor and inspiratory pressures of 60–70 cm H20 may be necessary to achieve tidal volumes of 400 to 500 ml. The term IPPB (intermittent positive-pressure breathing) implies positive pressure only during the inspiratory phase of ventilation. During the expiratory phase transtracheal pressure falls to zero. When alveolar instability is present, this fall to atmospheric level in the expiratory phase facilitates alveolar collapse and a subsequent fall in functional residual capacity. The use of 5–10 cm H20 (PEEP) may prevent this and reduce pulmonary edema by increasing intra-alveolar pressure. In our series of patients, PEEP was employed, but in the presence of very severe pulmonary pathology was of little value.

Despite the salvage of some patients who had severe respiratory disease with intermittent hyperbaroxia, it became clear to us that the only means of helping the most ill patients was an extracorporeal source of oxygen such as the membrane oxygenator. Our laboratory experiments (7–9) over the past three years have indicated that venovenous bypass using the membrane oxygenator may be of help before the proliferative phase of pulmonary oxygen toxicity involves more than 50 percent of functioning tissue.

Figure 4 is a description of the course of a patient who developed an aspiration pneumonia following an overdose of heroin and was treated at another hospital for four days prior to admission. During the next nine days, as pulmonary compliance decreased, increasingly higher inflation pressures and oxygen concentration were needed to maintain a satisfactory $Pa_{O_2}$. On the thirteenth day he developed bilateral pneumothoraces and appeared near death. He was transferred to the hyperbaric chamber for extracorporeal oxygenation. At 2 ATA, using venovenous perfusion, he was placed on the membrane oxygenator. Once flow rates of 2 L/min were reached, his $Pa_{O_2}$ remained at acceptable levels and he regained conciousness. Minute volumes and inflation pressures were greatly reduced and ventilation was maintained with air (the equivalent of an $F_{IO_2}$ of 40 percent at 1 ATA). After 24 hours the drainage from the right chest tube increased. After 31 hours on the oxygenator weaning was unsuccessful. Bleeding from the chest became severe. He was taken off bypass and the heparin was reversed with protamine. Once off the oxygenator, his $Pa_{O_2}$ dropped precipitously, and he expired. Postmortem examination of the lungs confirmed the diagnosis of far advanced oxygen toxicity, showing virtually all lung tissue to be in the proliferative phase with severe fibrosis.

We have concluded that if, after meticulous attention to ventilatory care including a trial of PEEP, maintenance of a normal or slightly elevated hematocrit, normothermia or mild hypothermia, normal acid-base balance, and intermittent hyperbaroxia, no improvement occurs, the use of extracorporeal oxygenation should be considered.

## Conclusions

Of the thirty patients in our series, eight survived, five of these probably because of the use of intermittent hyperbaroxia. The use of corticosteroids appears to slow the development of pulmonary oxygen toxicity. However, even the use of the hyperbaric chamber and corticosteroids could not salvage the majority of patients. In the future, extracorporeal oxygenation by means of a membrane oxygenator may provide the best physiologic milieu in which to keep patients alive while the severely diseased lungs are left to rest and, hopefully, heal. It is hoped that this paper will alert physicians who treat patients with severe acute respiratory disease to the dangers of prolonged inhalation oxygen therapy.

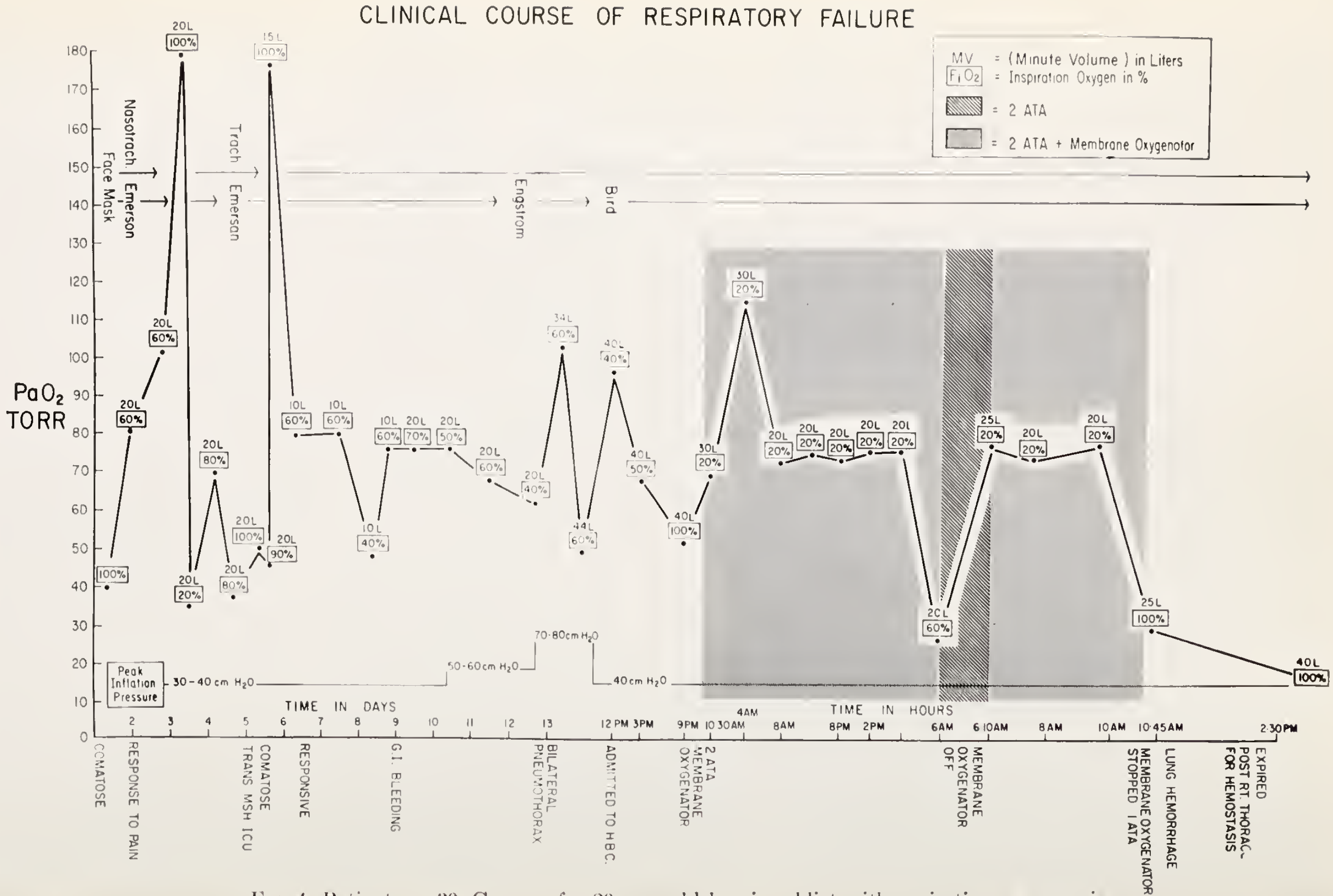

FIG. 4. Patient no. 30. Course of a 20-year-old heroin addict with aspiration pneumonia. Treated with conventionally administered oxygen, hyperbaroxia, and, finally, a membrane oxygenator.

## Summary

This study is a retrospective survey of patients sent to the hyperbaric chamber of the Mount Sinai Hospital, New York City in severe respiratory failure. Of thirty patients only eight survived, five probably because of the use of intermittent hyperbaroxia. One patient was treated by means of extracorporeal oxygenation with a membrane oxygenator for thirty-six hours. Postmortem examination of his lungs indicated that oxygen toxicity had already progressed to the irreversible proliferative phase. Thirteen postmortem examinations in patients who had had prolonged oxygen therapy revealed toxic pulmonary lesions described as exudative, intermediate and proliferative. The use of corticosteroids may prevent the development of the proliferative phase. The etiology and prevention of pulmonary oxygen toxicity is discussed. Extracorporeal blood oxygenation by means of venovenous bypass and a disposable membrane oxygenator may be used to treat acute respiratory failure in patients where there is insufficient functioning lung tissue to support life or where pulmonary oxygen toxicity has been superimposed on the original disease process.

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# Legg-Calve-Perthes Disease: A Statistical Evaluation of 358 Cases

JACOB F. KATZ, M.D.

A statistical analysis of our treatment experiences in 358 children with Legg-Calve-Perthes disease mainly at the Blythedale Children's Hospital furnishes the main theme for this presentation.

### *Associated Conditions*

There were fourteen children with classical Legg-Calve-Perthes disease in whom ten different concomitant medical conditions existed. These patients were not excluded. As shown in Table I, the associated problems included examples of Down's syndrome, cerebral palsy, cavus feet, and primary coxa vara. It was especially noteworthy that two of the children had diabetes mellitus discovered subsequent to the diagnosis of the hip involvement. The cycle of healing was not influenced by the secondary conditions.

TABLE I

*Conditions Associated with Legg-Calve-Perthes Disease*

| | |
|---|---|
| Bilateral cavus feet | 1 |
| Unilateral coxa vara | 1 |
| Mental retardation | 1 |
| Diabetes | 2 |
| Hyperthyroidism | 1 |
| Mild tibia vara | 2 |
| Down's syndrome | 1 |
| Cooley's anemia | 1 |
| Fractured shaft femur | 3 |
| Cerebral palsy | 1 |

### *Incidence by Age and Sex*

In the group of 358 children, there were 296 males (83 percent) and 62 females (17 percent). Regrouping by ages at onset, it was found that the largest number (35 percent) fell into the five- to six-year age range, valid for both sexes (Fig. 1). The left hip was involved in 44 percent of the children, the right hip 47 percent and both hips in 9 percent (Fig. 2).

### *Bilaterality*

An interesting observation came out of the analysis of bilateral cases. Most of them at time of diagnosis appeared to have been present together for an

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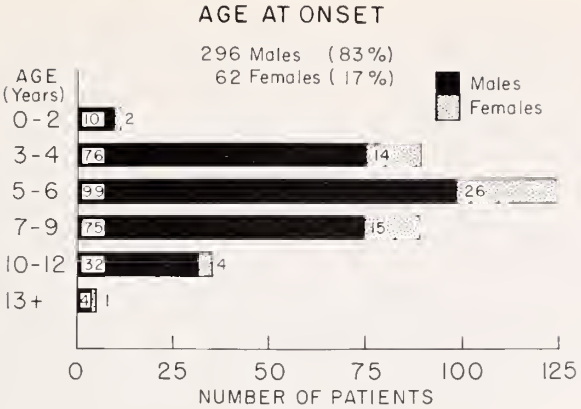


Fig. 1.

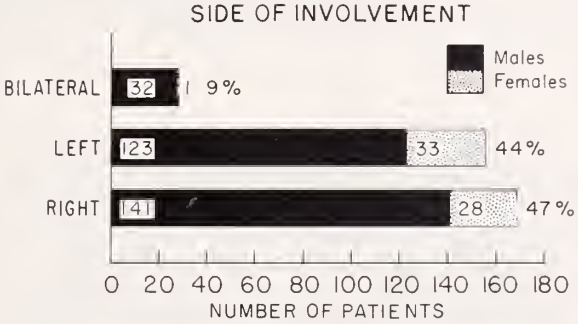


Fig. 2.

indeterminate period with usually the side of more recent onset being symptomatic (Fig. 3). Two other variations were seen occasionally. In one type, three youngsters under therapy for one-hip involvement developed second-side disease before the initial disease had fully healed (Fig. 4). In the other, three children had undergone full healing of one involved hip and then after an interval of well-being (usually about six to nine months) second-side involvement appeared and treatment had to be reinstituted.

*Racial Incidence*

The majority of children with LCP disease were white with an incidence of 13 male blacks (4 percent of 296) and one female black (½ percent of 62). The overall incidence of black children (excluding sickle-cell hemoglobinopathy and other mechanisms producing avascular necrosis) was 4 percent of 358.

*Minimal Disease Category*

In previous reports (1, 2) the author has made references to two related observations. One was existence of a variant of Legg-Calve-Perthes disease called minimal or abortive (Fig. 5), which presented clinically with classical

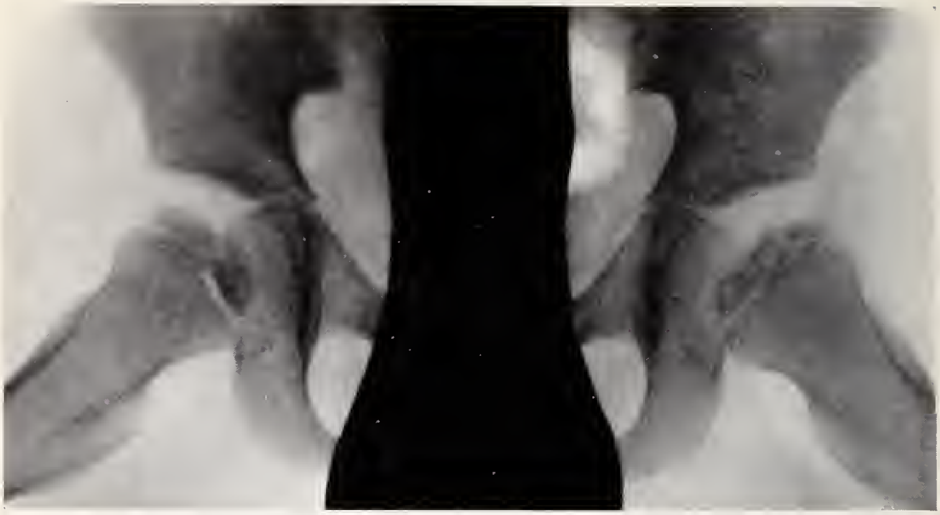


Fig. 3. Six-year-old white male child with bilateral Legg-Calve-Perthes disease diagnosed during investigation of unilateral hip complaint.

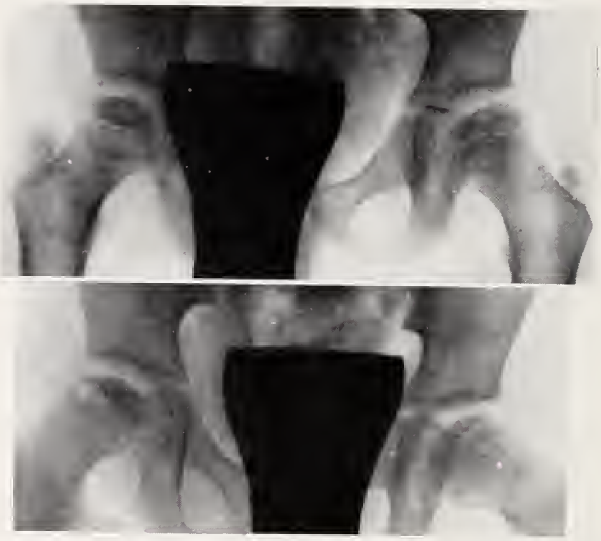


Fig. 4. Eight-year-old white male child who developed Legg-Calve-Perthes disease on the right side just prior to the completion of the treatment program for the left side.

limp or hip pain. These children always recovered with excellent reconstitution of the minimally involved hip. There were 21 such instances (6 percent) in the current series (Fig. 6). In this minimal category, three of the males were black. The second situation involved the accidental discovery of minimal or abortive involvement of the second side while the patient was being treated for unilateral classical disease (Fig. 7). Here, the minimally involved hip was not symptomatic and it also progressed to excellent restoration. There were 62 children

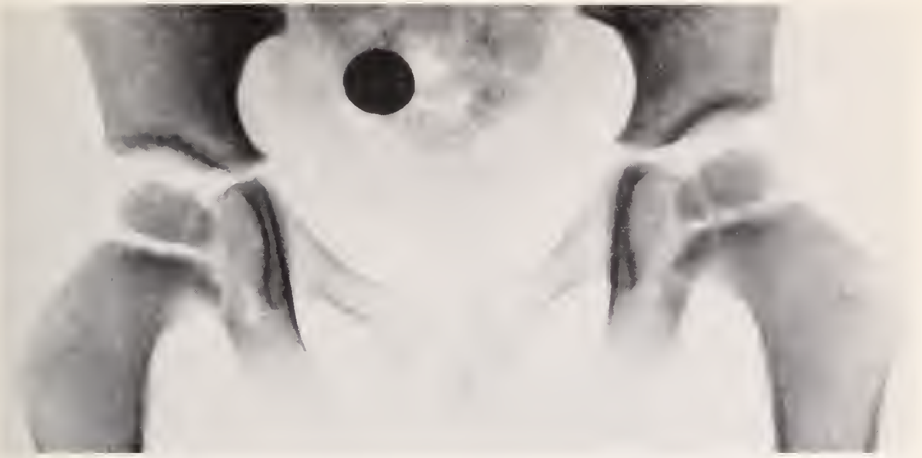
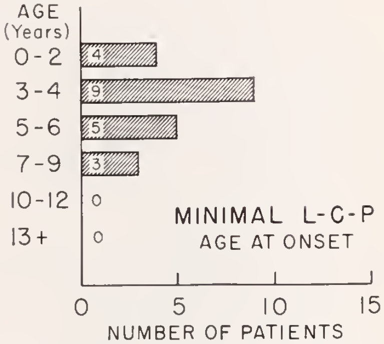


FIG. 5. Four-and-a-half-year-old black male child with evidence of scalloped shallow rarefactions in the subarticular area of both femoral capital epiphyses. This kind of radiographic presentation has been called minimal or abortive Legg-Calve-Perthes disease. It may also be unilateral. It has an excellent prognosis with or without treatment.

FIG. 6.



(17 percent) with asymptomatic second-side minimal hip changes. The right hip was affected 36 times in the minimal process (58 percent) and the left hip 26 times (42 percent). Commonly, both forms of minimal involvement occurred more frequently in the three- to four-year age group.

### *Order of Birth*

In 107 children, data was available of their order of birth. Forty children (37 percent) were firstborn; thirty-one (29 percent) were secondborn; twenty-one (20 percent) were third in succession; eight (7 percent) were fourth in birth sequence, and in the remaining seven (7 percent) there were instances of fifth, sixth, seventh, and eighth places in birth rank (Fig. 8).

### *Birth Weight*

In a small sample of forty-three children (38 boys and five girls) birth weight, length of gestation, and type of delivery were available. There were two

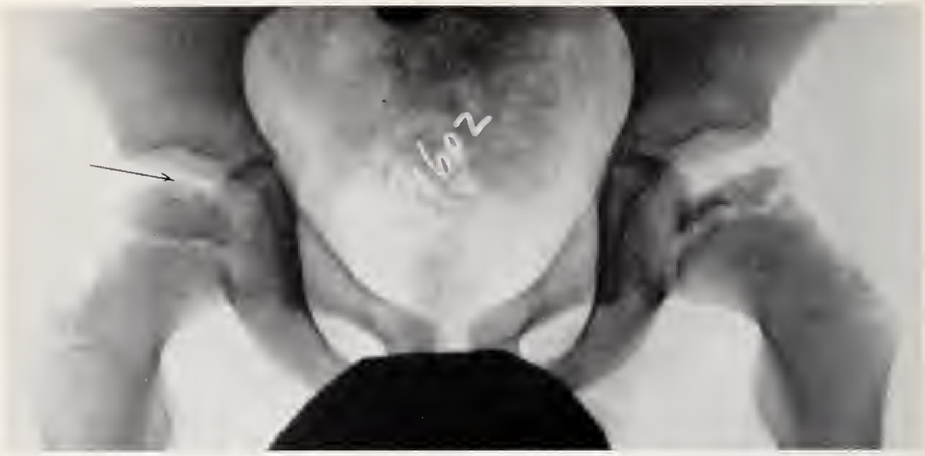


Fig. 7. Almost six-year-old white male child whose clinical involvement of Legg-Calve-Perthes disease of the left hip was confirmed with classical radiographic changes. An incidental finding was central minimal subarticular irregularity in the asymptomatic right hip.

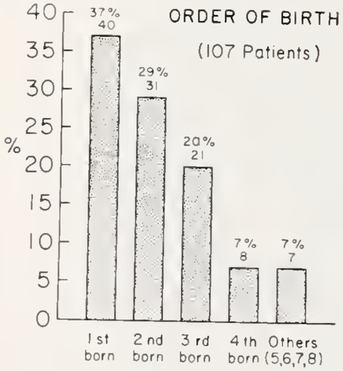


Fig. 8.

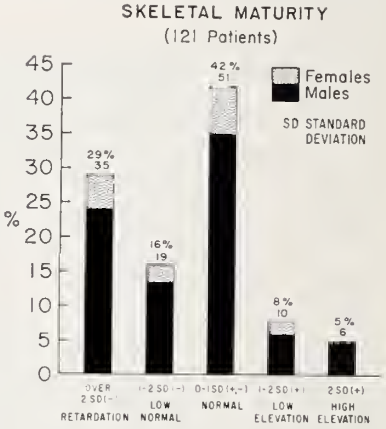


Fig. 9.

children with low birth weight (under 5½ lbs) who were full-term. There were three under five pounds who were premature at seven-month gestation levels. There was one caesarian section. All of these were males.

*Family History*

History of familial occurrence was noted in one of several relationships. There were five sets of sibling incidences: one in two sisters; one in brother and sister; and three in two brothers. None were twins. One of the brother combinations included brother and half brother (common mother). None of these children had known parent involvement. Two female children had mothers with Legg-Calve-Perthes disease, one of the latter also had an affected uncle. One female child and one male child had affected fathers (the latter also at Blythedale Children's Hospital in his childhood). One male youngster with LCP disease had an identical twin who was not affected. This familial association involved less than 3 percent of the entire study.

### *Skeletal Maturity*

In 121 children, carpal bone roentgenograms were available for skeletal maturity evaluation. (Fig. 9). Eighty children (66 percent) were in the group with less than two standard deviations above or below optimum development. Thirty-five patients (29 percent) were retarded in skeletal age more than two standard deviations. Six children (5 percent) were advanced in their skeletal maturity more than two standard deviations.

### *Development*

A limited opportunity was present to evaluate height and weight as developmental parameters in children with Legg-Calve-Perthes disease. The height and weight standards currently in use at the Mount Sinai Hospital were adopted for this study. Female children were represented insufficiently numerically (height, 9 cases; weight, 12 cases). Their height and weight generally fell within 2½ standard deviations of normal. The male children had better representation (87 cases). Although their grouping placed most within 2½ standard deviations of normal growth in height, twenty-one (24 percent) were below 2½ standard deviations (Fig. 10). Ninety-two boys had weight data (Fig. 11). Most fell within 2½ standard deviation parameters. An equivalent small number, about six, were above and below these boundaries.

### *Clinical Onset*

Three hundred children had recorded modes of clinical onset (Fig. 12). The largest number represented one hundred and twenty-nine children (43 percent)

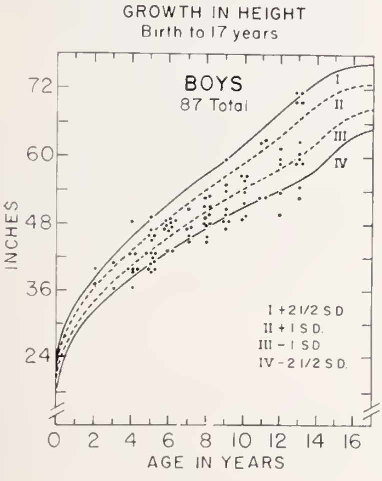


Fig. 10.

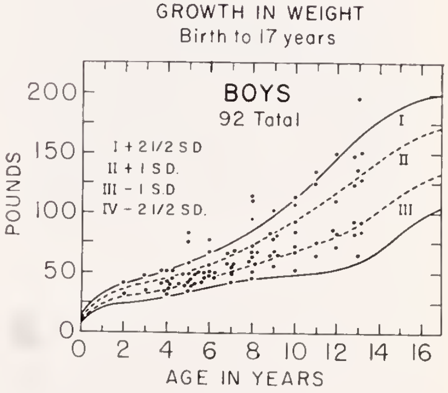


Fig. 11.

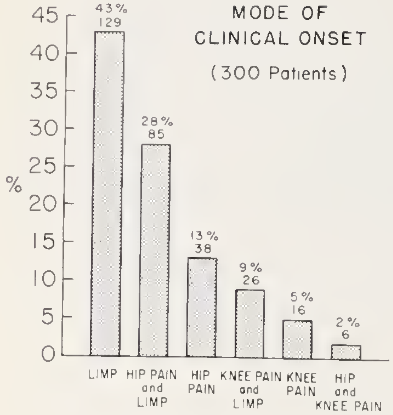


Fig. 12.

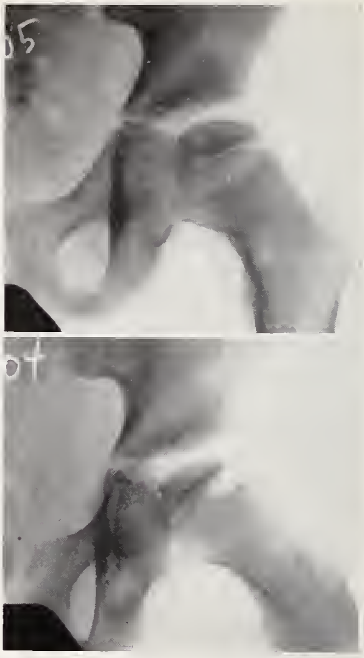


Fig. 13. Six-year-old black male child with Legg-Calve-Perthes disease of the left hip in the initial stage. There is diminution in overall size of the left femoral capital epiphysis without fragmentation.

presenting only with limp. Eighty-five youngsters (28 percent) had hip pain in addition to their limp. Thirty-eight (13 percent) complained only of hip pain. Twenty-six (9 percent) instances of knee pain and limp were found. Pain only in the knee was present in 16 children (5 percent). Six patients (two percent) complained of hip and knee pain.

### *Trauma*

Fifty-seven children (16 percent) had history of some associated injury, usually of minor severity. There were four youngsters in whom the discovery of LCP was pure chance; three of these children were being evaluated for effects of immediate severe acute injury; one was being checked for anomalous development. All four were asymptomatic prior to the unrelated incident requiring roentgenograms.

### *Radiography*

There were twenty-four occasions (7 percent) when clinical signs and symptoms warranted the diagnosis of Legg-Calve-Perthes disease but initial films were negative. Sixteen of these children had positive diagnoses on repeat roentgenograms within four months; the remaining eight were diagnosed on roentgenograms taken from five to eleven months after the initial films. Although the likelihood of failing to find roentgenographic changes on initial examination is not great (seven percent in this study), my practice is to repeat the films at intervals up to six months before conceding that the symptoms had other causes, usually transient synovitis.

The roentgenogram has become a key tool in evaluating many phases of Legg-Calve-Perthes disease. In a previous report (3) the discrepancy between the stage of the disease and the onset of symptoms had been noted. The disease process then had been arbitrarily divided into three prominent stages: Initial (0–6 months) (Fig. 13); Fragmentation (6–12 months) (Fig. 14); Reparative (over 12 months) (Fig. 15). In the present series, at the time of diagnosis in 286 patients, there were 121 children with initial stage disease, 51 with fragmentation and 114 with reparative changes. In a closer review of the initial stage, 37 patients (31 percent) were found with density of the proximal femoral capital epiphysis (Fig. 16), 25 patients (21 percent) with subchondrial lysis (crescent sign), and 20 (17 percent) with a combination of density and subchondral lysis

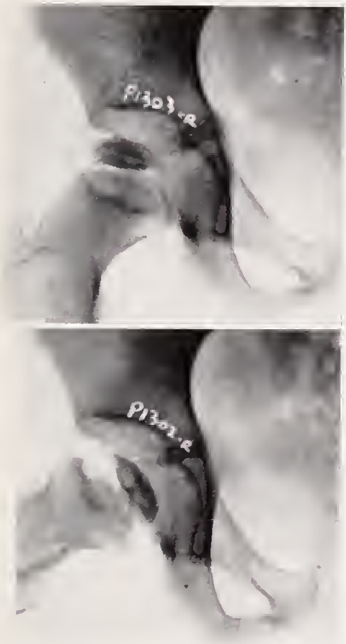


FIG. 14. Five-year-old white female child with history of right-sided limp slightly over six months prior to present radiograph.

There are minimal marginal changes with early lucencies in the generally dense, smaller right femoral capital epiphysis consistent with early fragmentation stage.

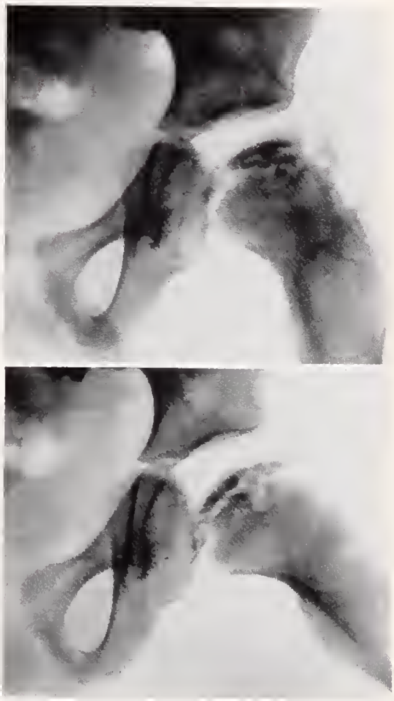


FIG. 15. Seven-and-a-half-year-old white male child whose symptoms of left hip discomfort went back one year. Diagnosis was not made radiographically until four months prior to present film.

In addition to fragmentation of the left femoral capital epiphysis, there is obvious marginal new bone formation consistent with the reparative stage.

(Fig. 17). Including all stages, subchondral lysis was present only 16 percent of the times. Although conceded to be a reliable early finding when present, it is not found in most films at time of diagnosis.

### *Mode of Treatment*

Until two years ago the treatment for Legg-Calve-Perthes disease at Blythedale Children's Hospital had been a form of nonweight-bearing management. This varied from crutches and sling, wheel chair, bed rest and traction, to abduction brace. For the past two years the service policy has adopted an abduction brace designed at the hospital (Fig. 18) to permit weight-bearing. Although the statistics concerned with end results will include all modalities of therapy, obviously the larger number of nonweight-bearing cases will have the greatest influence.

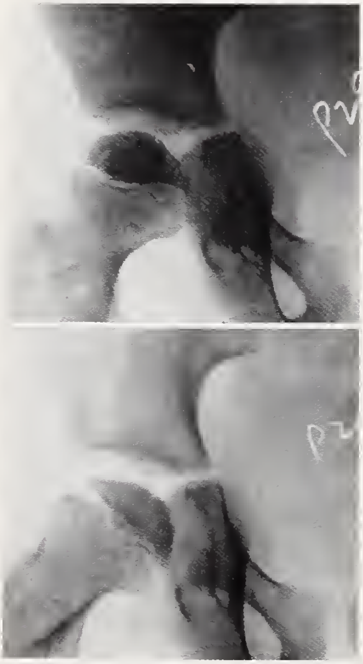


Fig. 16. Six-and-three-fourths-year-old white male child with pain and limp in the right hip of about four months duration.

The right femoral capital epiphysis is dense, especially prominently in the antero-posterior view.

The Legg-Calve-Perthes disease is in the initial stage.

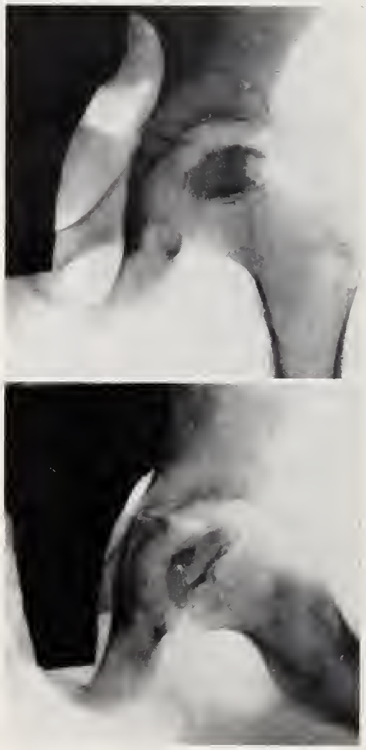


Fig. 17. Six-year-old white female child whose symptoms of left-sided limp and thigh discomfort started about three months prior to this radiograph.

The left femoral capital epiphysis is dense (shown better in the antero-posterior view). There is also anterior subchondral lysis (crescent sign) of the capital epiphysis (seen in the frog-lateral view).

### *Method of Determination of End Result (Fig. 19)*

In previous reports (3–5) two major methods of end result evaluation were described. One consisted of the Heyman and Herndon calculation of a variety of quotients (epiphyseal, acetabular, etc.) leading to a comprehensive quotient. This method attempted to quantitate by percentage the degree of distortion of the affected hip in comparison to the unaffected side. The other was the Knud

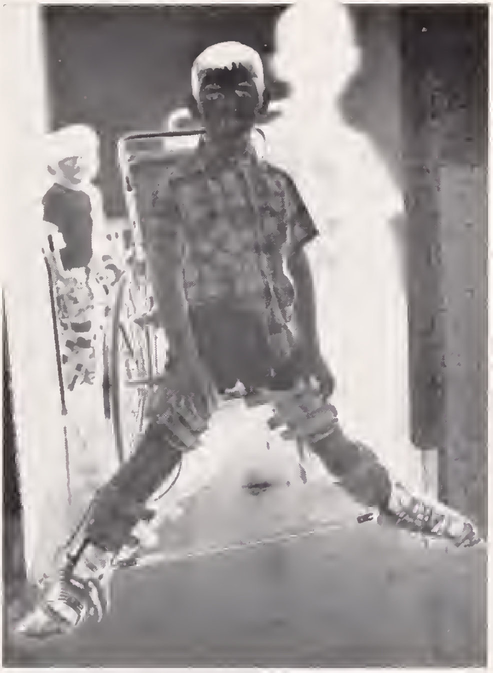


Fig. 18. Youngster with Legg-Calve-Perthes disease under treatment at the Blythedale Childrens Hospital with an abduction brace permitting weight bearing.

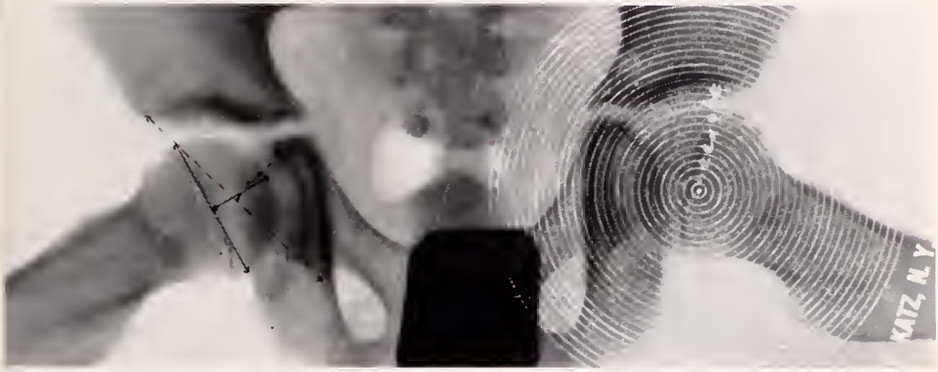


Fig. 19. This radiograph indicates parts of two of the systems of measurements used in determining the end result of Legg-Calve-Perthes disease.

The lines drawn over the right hip are part of the calculations (epiphyseal and acetabular indices) of the Heyman and Herndon method; the template of concentric circles on the left hip is an overlay for the determination of sphericity by the Knud Mose method.

Mose method of defining the sphericity of the restored femoral capital epiphysis by using an overlay of a transparent template of ruled concentric circles. The epiphyseal quotient (part of the Heyman and Herndon calculation) was also included in the final assessment.

*End Result*

The Knud Mose method of determining the end result is used in this report. In all calculations of end result data to follow, the twenty-one patients with minimal disease have been excluded. Since they reconstituted reliably almost perfectly, I felt it unfair to use them to weight the general statistics.

With this exclusion, there were 317 patients on whom end result data was available. 123 children (39 percent) ended their treatment period with good results, 123 (39 percent) with satisfactory results and 69 (22 percent) with poor results. The good and satisfactory categories were regarded to be acceptable, making this combined group 78 percent and the unacceptable group 22 percent.

Using this general base, all elements that might have had a modifying influence on this result were isolated and examined. Included here were such factors as: age, kind of treatment, stage of disease at onset, type of epiphyseal involvement, metaphyseal cyst, subluxation, and duration of therapy.

*Age*

Analysis of the end result data by age groups (Fig. 20) showed dramatically the effect of age on the quality of the final result. In ages up to six years, the percentage of poor results was less than half that of the inclusive figure of 22 percent for the entire group. Beginning at age seven, poor results exceeded the average for the entire group and continued to worsen with advancing age.

*Kind of Treatment*

In the treatment of Legg-Calve-Perthes disease, the oldest experience at Blythedale Children's Hospital was in miscellaneous methods of nonweight-

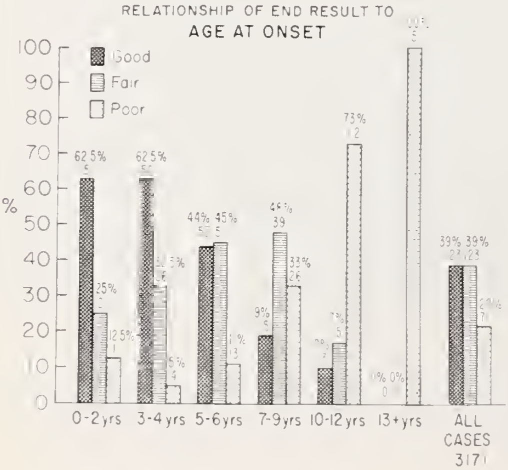


Fig. 20.

bearing. This included crutches and sling, caliper weight-relieving braces, wheel chair, bed rest, and plaster immobilization. Later this was replaced with exclusive use of bed rest and traction. This in turn yielded to bed rest with abduction brace and finally, in the past two years, to abduction brace with weight bearing. Interspersed, through the years, was a small scatter of surgically treated patients.

Review of data compiled in Figure 21 shows that in 316 patients on whom information was available, the two largest groups were treated with bed rest and traction and abduction brace without weight-bearing. The abduction brace group had 19 percent poor results in comparison to 26 percent for those on bed rest and traction. The two other categories noted in the diagram, miscellaneous and surgical, must be viewed with caution since they include too few patients at this time. Neither of the two larger groups offered significant advantage when compared with the overall end result of 22 percent poor results.

*Stage of Disease at Onset*

Two hundred and eighty-six patients had sufficient data to permit identification of the roentgenographically defined stage at onset (Fig. 22). In the one

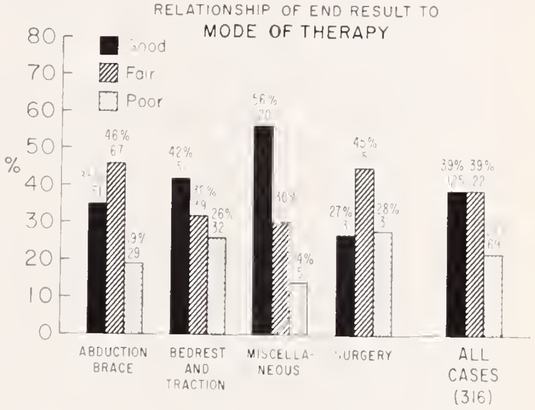


Fig. 21.

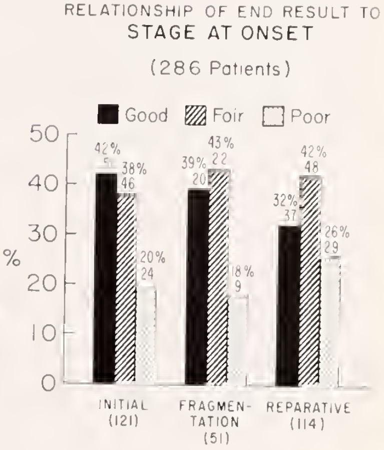


Fig. 22.

hundred twenty-one children in the initial stage, 20 percent ended with poor results and 80 percent with acceptable results. In fifty-one children in the fragmentation stage, 18 percent had poor results and in one hundred and fourteen patients in the more advanced reparative stage, 26 percent had poor results. It is quite evident here also that insignificant advantage is derived from earlier identification of the Legg-Calve-Perthes disease when results of therapy are compared.

### *Type of Epiphyseal Involvement*

Almost since the earliest description of this disease, the thesis has been supported that differences existed in children with LCP disease in the way they progressed to healing. Legg (6) felt he was unable to influence the course of healing in his cases but he was confident that he could distinguish early between the better prognosis "mushroom type" and the poor prognosis "cap type." With O'Garra's (7) observations that roentgenograms could be used to differentiate "whole head" from "partial head" involvement, and with confirmatory studies from Ponseti (8) and Goff (9) of different healing patterns, the basis of varying prognosis seemed more secure. Catterall most recently (10) divided his cases into four groups, the prognosis worsening in groups 3 and 4. He felt that in group 1 only the anterior part of the epiphysis was involved without collapse and without significant deformity. His best results fell into groups 1 and 2.

In 212 cases in this study, it was possible from x-ray films to estimate the presence and degree of radiographic intactness during healing. The purpose was to identify "whole head" versus "partial or segmental head" involvement.

I found that where portions of the proximal femoral epiphysis remained intact, it was usually in the posterior medial segment. It required two roentgenograms, one taken in the A-P projection with the legs internally rotated and a second in the frog-lateral view to adequately identify this area. Difficulties encountered in this portion of the study fell into two categories: firstly, films of poor quality or nonstandard positioning; and secondly, films taken over an insufficient time span to show the evolution of the cycle of healing (Fig. 23).

When all of the epiphysis took part in fragmentation and resorption the category was obviously "total" or "whole head" (Fig. 24). In the group of partial or segmental epiphyseal involvement, three grades were defined varying with the size of the unaffected portion of the epiphysis. The "frog-lateral" roentgenogram usually was the more important one in arriving at this estimate.

Grade I was defined arbitrarily as that in which the remaining epiphyseal fragment encompassed one-fourth or less; Grade II was between one-fourth to one-third (Fig. 25), and Grade III, one-third and over of the entire epiphysis (Fig. 26).

Occasionally it was difficult to be sure whether a tiny marginal fragment represented Grade I intactness or early regeneration of a "total head" variety (Figs. 27 and 28).

Figure 29 graphically demonstrates the division into these four groups of 212 patients on whom data was available. There were fifty-eight patients (eleven

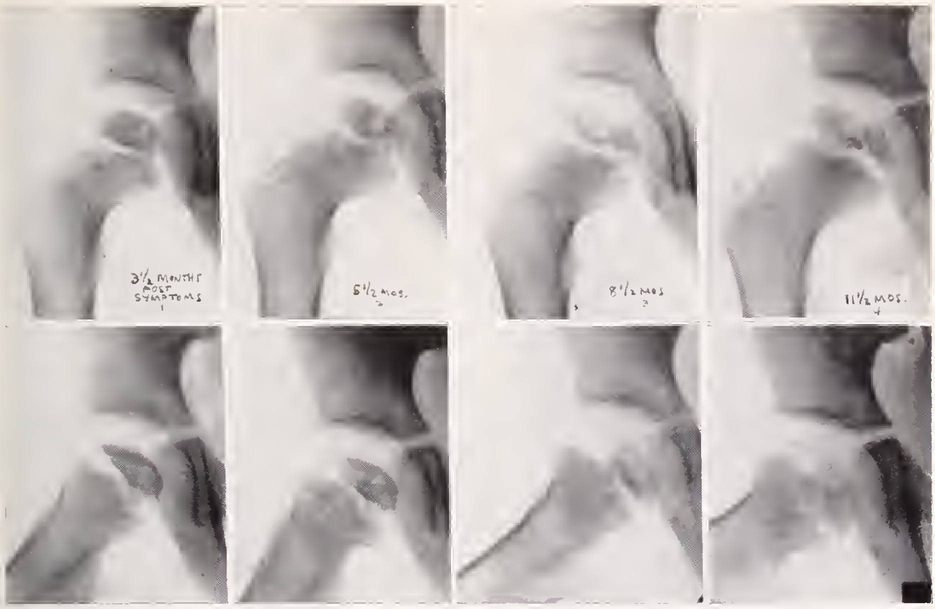


FIG. 23. These serial radiographs of a child almost five years old with Legg-Calve-Perthes disease of the right hip indicate the progressive rarefaction of the proximal femoral epiphysis. The resorptive mechanism continues until the stage at eight and a half months at which time an estimate can be made that the disease is segmental in character and Grade II in severity (retained epiphyseal fragment ¼ to ⅓ of the whole). At eleven and a half months, there is a reversal of the process with ossification now contributing to the healing process.

females) with total head involvement (27½ percent). In Grade I, there were also fifty-eight patients (fourteen females) making up 27½ percent of the total. There were seventy-one patients (thirteen females) in Grade II (33 percent) and twenty-five children (one female) in Grade III (12 percent). The gross characteristics of this division are the approximately equivalent numbers (boys and girls) in the total, Grade I and Grade II groups. The best prognosis Group III category is smaller than the other three, and it has a smaller number of female children.

In Figure 30, the classification of kinds of Legg-Calve-Perthes disease is related to the end result in each type. The highest percentage (29 percent) of poor results was found in the total head group (58 patients). In Grade I and II (71 patients) the poor results were almost identical 17 percent and 18 percent respectively. In Grade III (25 patients) the percentage of poor results (12 percent) was lowest and the percentage of good results (72 percent) the highest. This relationship is in agreement with the consensus in the literature that partial head involvement has a better prognosis.

Although my classification is labelled differently and is derived differently than Catterall's (10), the unmistakable association of better results with the milder form of epiphyseal involvement and poorer results in the totally involved epiphysis is consistent.

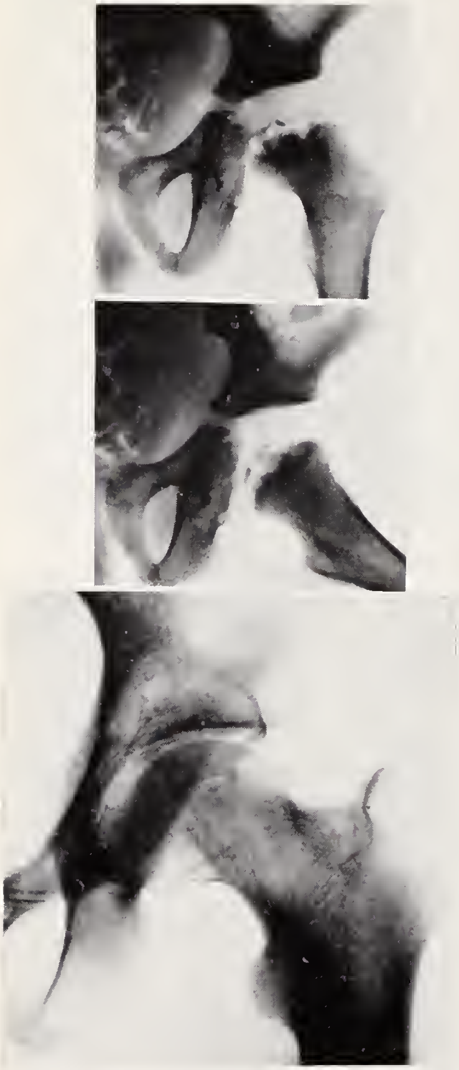


Fig. 24. Four-year-old white male child with history of left sided limp for about one year prior to the present radiograph. The legg-Calve Perthes involvement of the left hip is obviously of the "whole head" variety. The fragmentation and "wafer" thinness are characteristic changes.

The follow-up radiograph taken fifteen years later shows satisfactory restoration of the articular contour and congruence.

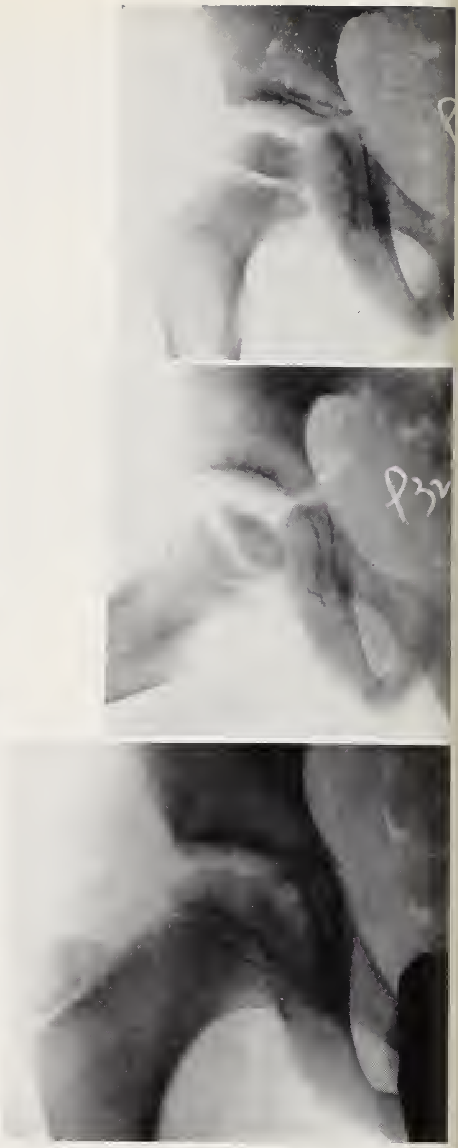


Fig. 25. Four-and-a-half-year-old white male child with Legg-Calve-Perthes disease of the right hip. His initial radiographs three weeks after onset of symptoms do not permit evaluation of the type of involvement present. The serial radiographs in Fig. 23 belong to this patient and it is clear that it may take eight or more months before identification of the end of the resorptive phase is possible. (Grade II).

Follow-up radiographs four and a half years later show excellent reconstitution.

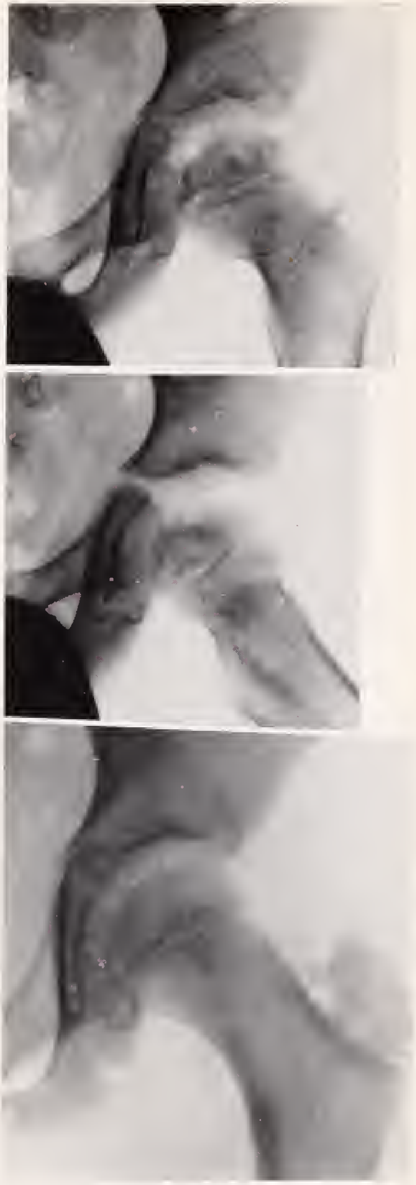


FIG. 26. Six-and-three-fourth-year old white male child with left-sided Legg-Calve-Perthes disease with symptoms of hip discomfort of six months duration.

This is segmental disease Grade III in severity (over one-third retained epiphysis).

Follow-up radiographs four and one-fourth years later show excellent restoration.

*Metaphyseal "Cyst"*

Investigators of Legg-Calve-Perthes disease frequently have described metaphyseal lucencies. Usually their connotation has been of altered poor prognosis and impaired end result.

Mindell and Sherman (11) noted asymmetric growth retardation or arrest as well as poor prognosis with extensive cyst formation. Edgren (12) noted poor healing of the femoral head, shortening and broadening of the neck and prema-

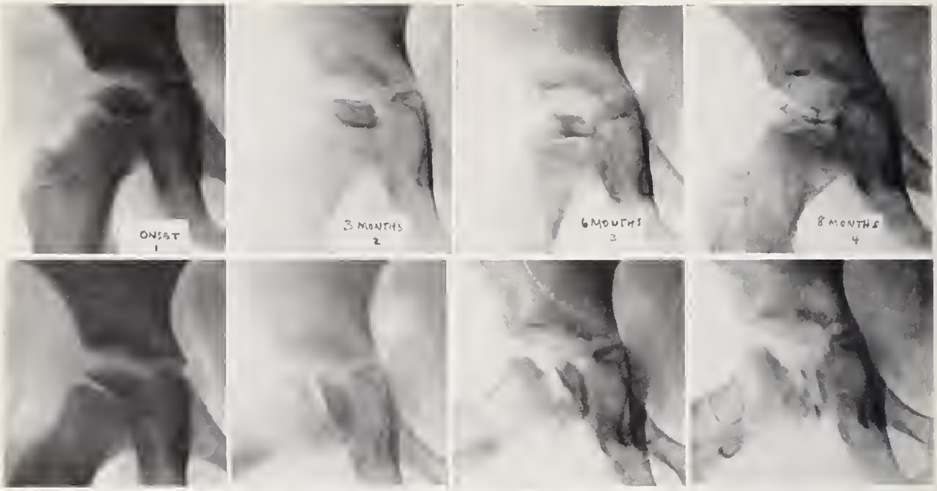


FIG. 27. Four-and-a-half-year-old white female child with Legg-Calve-Perthes disease of the right hip.

The first radiographs taken two weeks after limp developed represent initial stage of disease. The serial radiographs graphically represent progressive resorptive changes. At eight months after onset a questionable tiny postero-medial margin of intact epiphysis remains. Whether this is Grade I (less than one-fourth epiphyseal retention) or total head involvement seems academic.

The important point is that an erroneously optimistic estimate of the degree of segmental involvement might have been made if too few films were studied.

ture closure of the growth plate. Catterall (10) included diffuse metaphyseal reaction as one of the signs of the femoral "head-at-risk."

I think that metaphyseal "cysts" represent a stage in the repair of metaphyseal necrosis. In the series being reported, metaphyseal cysts were noted in one hundred and two patients (29 percent of all patients) (Fig. 31). Of these, 68 percent had acceptable and 32 percent poor results. Although my earlier impression had been that metaphyseal cysts did not add significantly to morbidity, this current analysis offered some confirmation of the consensus in the literature to the contrary (Fig. 32 a–b).

### *Subluxation*

This characteristic in the sequential changes of healing in some instances of Legg-Calve-Perthes disease has not received as much general recognition as others. When accorded proper weight it also has been in the direction of association with poorer prognosis.

Catterall (10) found that lateral subluxation contributed to the ultimate femoral head deformity by the mechanism of uneven pressure on the involved femoral segment with ultimate feed-back distortion of the acetabular margin. Somerville (13) felt that subluxation had the potential for marked deformity, if allowed to persist.

In this series there were thirty-four patients with subluxation (11 percent of

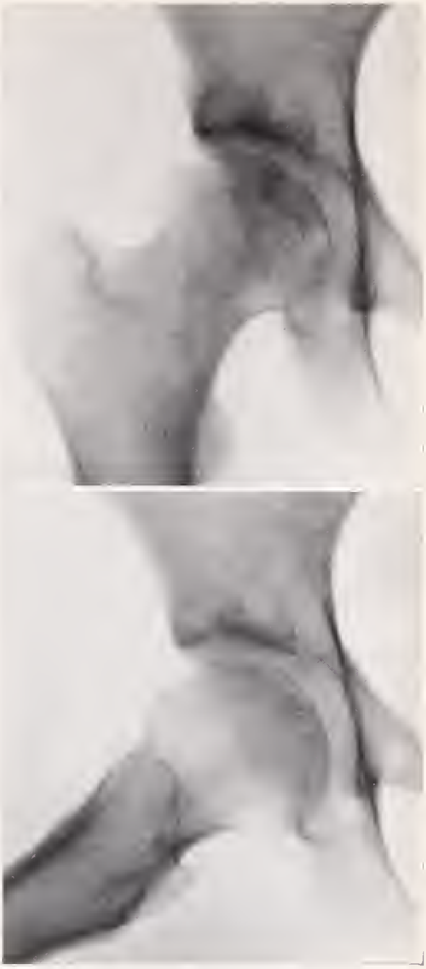


Fig. 28. These radiographs represent the follow-up of the child whose films appear in Fig. 27. They were taken eleven years after onset.

This is an acceptable result radiographically and clinically.

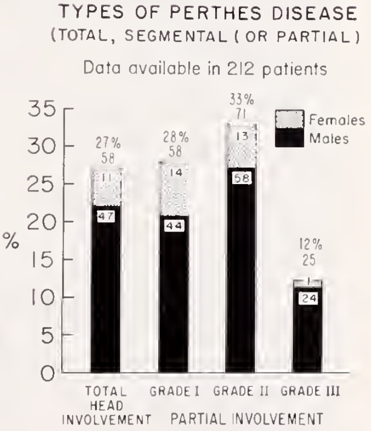


Fig. 29.

318 patients with whom this data was available) (Fig. 33). Twenty-eight of them were male (82 percent) and six female (18 percent), adhering to the usual incidence of 4:1 males seen regularly in unselected examples of Legg-Calve-Perthes disease. The largest group (sixteen patients) fell into the seven to nine year age group representing a shift from the five to six year age group preference of the larger unselected general group.

By arranging the subluxation patients into ages at onset, the largest number

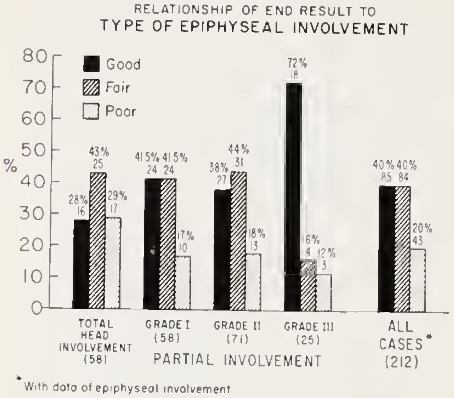


FIG. 30.

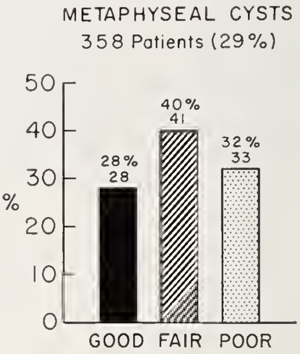


FIG. 31.

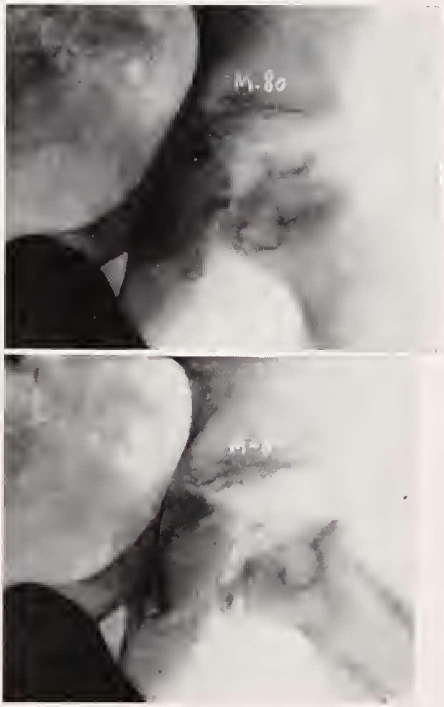


FIG. 32a. Ten-year-old white male child with symptoms of limp and groin pain approximately one year prior to the radiographs shown here. The left hip displays prominent metaphyseal lucencies in addition to marked epiphyseal fragmentation.

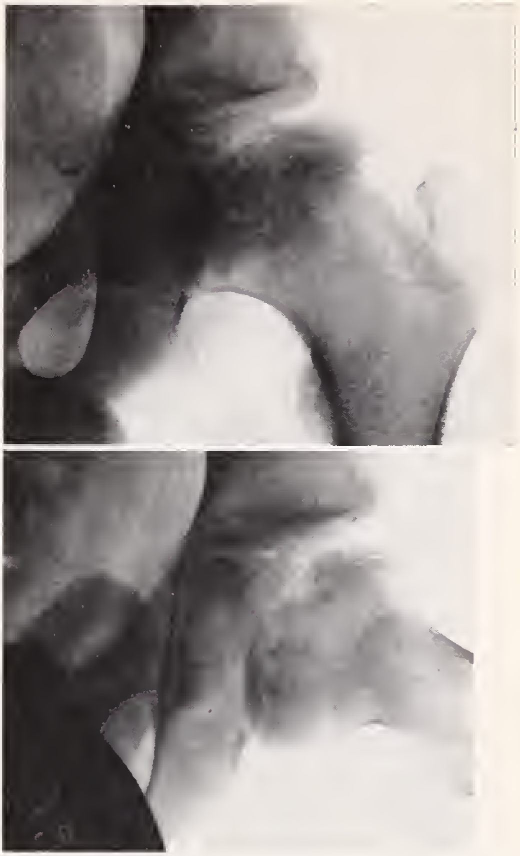


FIG. 32b. Radiographs two and a half years later present the distortion of "mushroom-like" ovoid configuration with an unsatisfactory result rating.

of poor results (ten) fell into the seven to nine year age group. All the patients in the ten to 12 age and 13+ groups had poor results (Fig. 34).

Additional parameters of this subluxation group were investigated. The types of femoral head involvement in thirty of these patients were determined (insufficient data in four children). Twelve children's hips fell into the total head group and eighteen into the partial or segmental grouping. Of the latter, six were Grade I (tiny unaffected segment), eleven were Grade II (medium unaffected segment) and only one in Grade III (large unaffected segment).

None of the total head type with subluxation yielded good results (Fig. 35); six became fair and six poor. The segmental subgroups tended to scatter with representations in each of the good, fair and poor categories (Fig. 36).

The conclusion arrived at from this section of the study is that subluxation is a marker for poor prognosis. The overall end result of 56 percent poor results in this category was significantly above the 22 percent found in the general end result statistics.

## *Duration of Therapy*

This phase of the Legg-Calve-Perthes disease experience has received considerable attention. It contributes a large part to the anxiety and distress of the

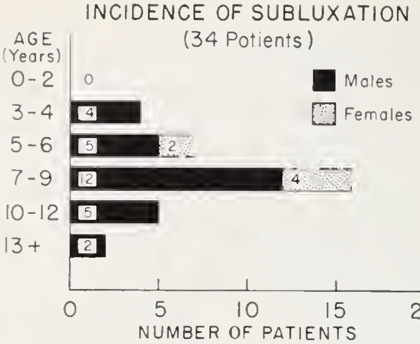


Fig. 33.

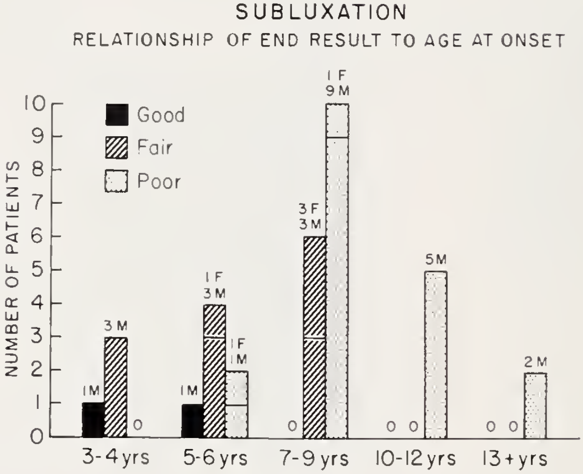


Fig. 34.

child and his family in the ongoing management of the disease. Despite its importance there is no agreement on which criteria should be used in the decision to stop the treatment.

For easy identification one approach had been to persist with therapy until complete roentgenographic reconstitution occurred. Despite the attraction of such a finite end point, two factors challenged this routine. One was the inordinately long period of time consumed from onset to complete restoration, occasionally three to five years; the other was the empiricism that such a prolonged interval was not required when the "weight-bearing area" (Goff) of the femoral capital epiphysis was restored. My practice has been to continue with treatment until all infarcted bone (sequestra) has been resorbed and significant peripheral new bone formation has taken place. Usually a small central residual area of lucency is present when treatment is discontinued. This is noted to fill in on subsequent follow-up roentgenograms, one or several years later (Fig. 37).

Analysis of data on duration of therapy was performed to determine whether relationships existed between it and age at onset, stage of disease at onset, type of involvement of femoral epiphysis, and final result. Three arbitrary divisions of duration of therapy were selected consisting of 1) under 1½ years; 2) 1½ to 2½ years; and 3) over 2½ years.

No significant difference occurred in duration of therapy at all ages at onset

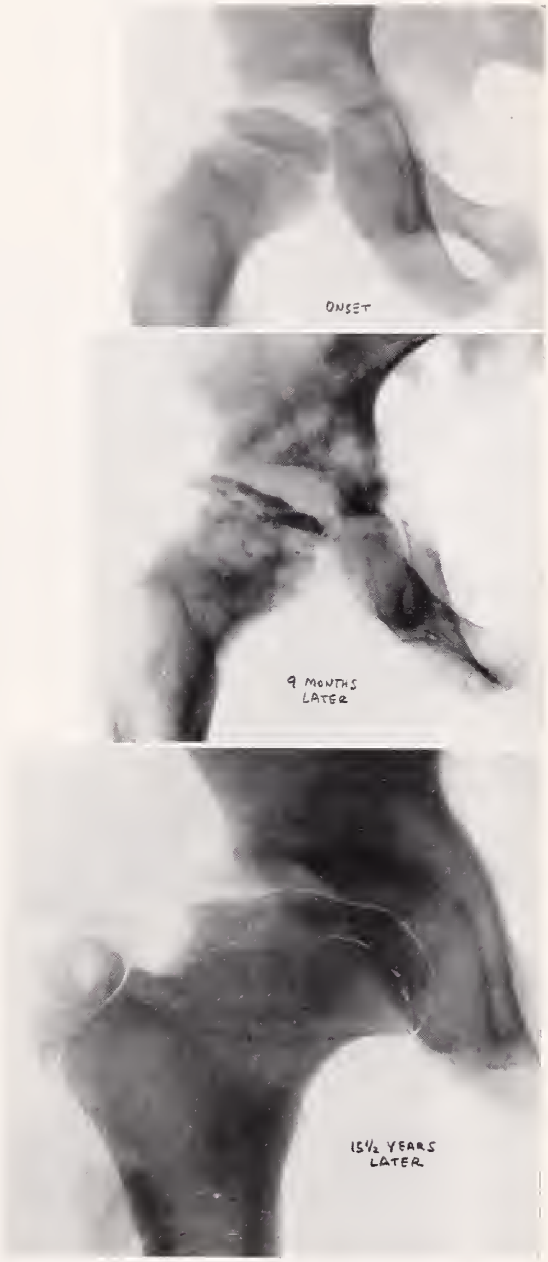


FIG. 35. Eight-year-old white male child whose symptoms of limp and knee and hip pain started three months before the initial radiograph shown here. Subluxation of the right hip was already evident with lateral and upward migration of the femoral head.

Nine months later there is progression of the subluxation (as well as of metaphyseal cysts and severe fragmentation and flattening of the epiphysis).

At follow-up fifteen and a half years later there is marked distortion of the femoral head with an unequivocally unsatisfactory radiographic result.

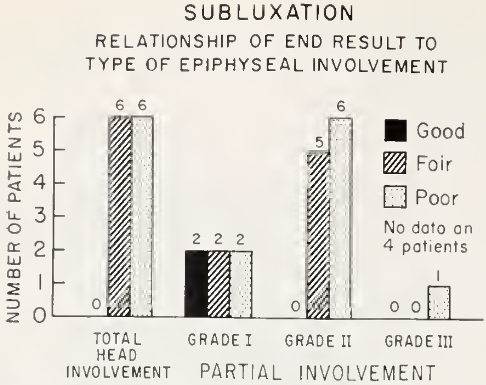


Fig. 36.

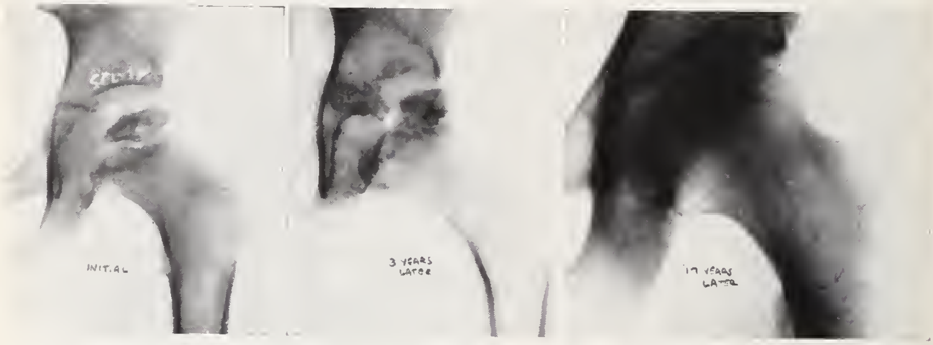


Fig. 37. Six-and-a-half-year-old white male child with symptoms of limp and hip pain on the left about six months prior to the initial radiograph shown here.

Almost three years later the radiograph shows good peripheral restoration with residual central lucency. Weight bearing was permitted.

Follow-up film seventeen years later shows good restoration. He is asymptomatic and performs without restriction.

beginning with the three to four year group. Too few patients were in the birth-to-two-year group to regard its variability to be significant (Fig. 38).

Data concerning relationship of duration of therapy to the stage at onset was examined (Fig. 39). There were no significant differences in the duration of therapy for those youngsters whose disease at onset was in the fragmentation or reparative stages. They had excellent correlation with the overall experience in which about half spent 1½ to 2½ years and one-fourth under 1½ years and one-fourth over 2½ years. Those children whose treatment program started in the initial stage were noted to have a smaller number treated under 1½ years and an equivalently larger number with 1½ to 2½ year time period.

The relationships between duration of therapy and type of disease were reviewed (Fig. 40). Children whose disease was of the Total Head variety had the least percentage of under 1½ years and highest percentage of over 2½ year treatment periods. Those with Grade I segmental involvement had a slightly smaller percentage of over 2½ year treatment time. Grade II and Grade III

Fig. 38.

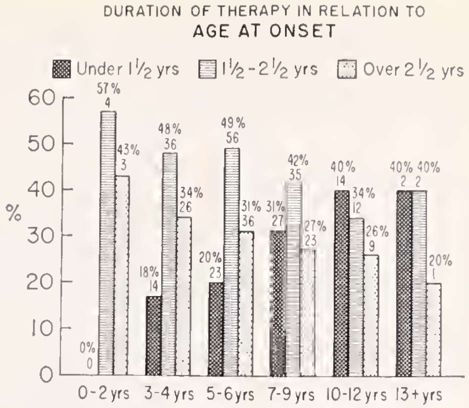


Fig. 39.

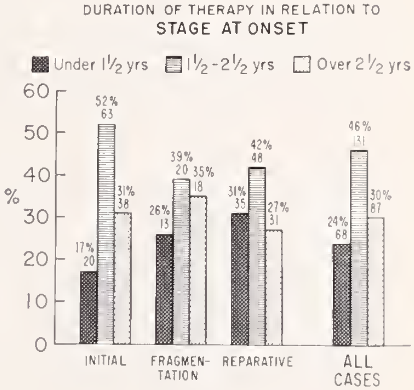
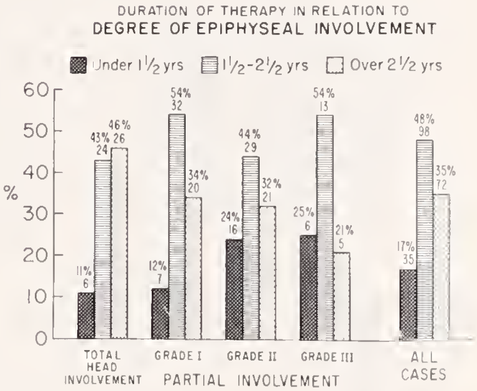


Fig. 40.



(more benign types of involvement by my rating) had higher percentages of under 1½ years treatment programs and equivalently smaller percentages of over 2½ year treatment intervals.

The final assessment in this sphere was the relationship between duration of therapy and end result (Fig. 41). No significant effect on the end result appeared to reside with duration of therapy. By coincidence, the percentage of patients in the three arbitrary divisions of time of treatment were practically identical in the good and poor end result columns. Obviously the answer to improvement of the quality of repair was not increasing treatment time.

*Range of Motion*

At termination of therapy it was found that over 50 percent of the children had less than 20 percent hip joint restriction in internal rotation and abduction.

*Leg Lengths*

The presence of leg shortening was noted in one hundred and forty-three children (Fig. 42). There were nine children (six percent) with one inch or more; thirty-two (22 percent) with one-half to one inch; one hundred and two, (72 percent) under one-half inch. Just as one might have anticipated, there was a specific relationship between the amount of shortening and the percentage of poor results. Under one-half inch shortening, poor result patients remained around 20 percent. At one-half inch it was 45 percent and above that it was worse.

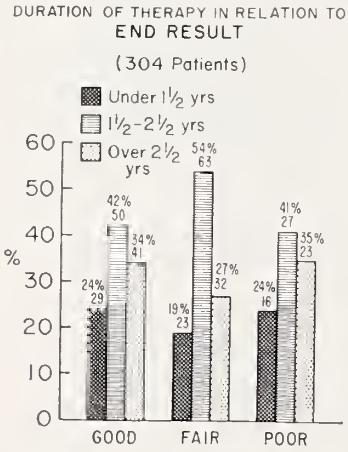


Fig. 41.

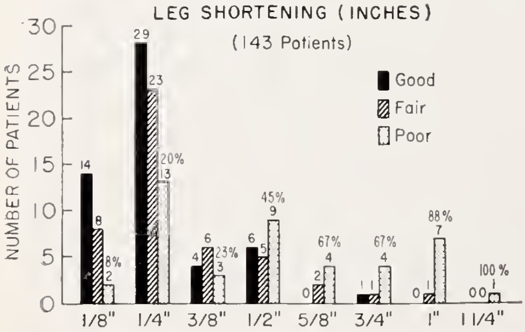


Fig. 42.

## Discussion

Our series was originally considered to be 387. Twenty-nine cases which were initially regarded as LCP were excluded from this study because their disease processes followed a known mechanism other than LCP disease (Table II), leaving the series of 358 cases which are analyzed in this paper.

Analysis of this clinical material, all personally observed and followed, has furnished considerable data on the characteristics, behavior, and immediate end result of 358 children with Legg-Calve-Perthes disease. Most of the patients received their treatment at the Blythedale Children's Hospital under uniform circumstances with the details of management varying with intermittent changes in program.

In many areas there is agreement with the usual experience, especially male distribution, equivalent unilateral involvements except for a small bilateral incidence and virtual confinement to white children. The four percent incidence of this disease in black children reported here may be slightly high since, in the earlier years of this study, hemoglobin electrophoretic determinations were not available. There is, however, a definite if small presence of Legg-Calve-Perthes disease in black children.

Attention has been directed to a minimal variant of Legg-Calve-Perthes disease whose place in the overall disease picture is not totally clear. Because these minimally affected children uniformly reconstituted almost anatomically they have been excluded from the statistics of treatment results. It was found that the remainder of the patients could be segregated into groups of apparently greater or lesser epiphyseal resorption, ranging from total head to degrees of partial head involvement. The value of this separation lay in offering a prognosis which was poorer for the totally involved head and best for the Grade III segmentally (least fragmented) involved head.

Age at onset remained a reliably good prognostic category with children under six years of age included in this protective zone.

TABLE II

*29 Cases Simulating LCP**

| | |
|---|---|
| Hypothyroidism | 3 |
| Sickle-cell disease | 8<br>(6 SS – 2 SC) |
| Gaucher's disease | 2 |
| Epiphyseal dysplasia | 5 |
| Postosteomyelitis | 2 |
| Postcongenital hip dislocation | 2 |
| Posttuberculosis | 1 |
| Arthritis | 1 |
| Posthip trauma | 5 |

* Not included in this study.

The presence of metaphyseal cysts and subluxation increased the chances for a poor end result, the latter markedly more than the former.

Nonambulatory nonweight-bearing modes of therapy, at least those in use up to several years ago, seemed to be equivalently effective. Experience is accumulating at present with a revised form of treatment using an abduction apparatus permitting weight-bearing.

Therapy still consumes large amounts of time with most children spending 1½ to 2½ years and over. No advantage seemed specifically assignable to longer periods of treatment or to the stage of disease at onset of therapy.

The quality of the clinical end result in terms of joint mobility and leg length discrepancy appeared to follow the roentgenographic parameters.

## Conclusions

Legg-Calve-Perthes disease remains challenging and unresolved. In the tabulation of *Knowns*, there are:

Male Incidence
White Dominance
Improved prognosis with younger age and milder epiphyseal involvement.
Worsened prognosis with older age, total head involvement, metaphyseal cyst formation and subluxation.

In the listings of *Unknowns* there are:

Uncertain etiologic associations with vascular anatomy, altered growth and development, trauma, familial background and heredity.
Lack of available prophylaxis
Absence of an ideal treatment modality for overall predictable effectiveness in the disease as a whole.

Continuing integrated studies with prospectively oriented guidelines offer the best opportunity for additional clinical information. Correlated experimental research will be required for the ultimate break through.

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# Suppurative Pylephlebitis and Liver Abscess Complicating Colonic Diverticulitis: Report of Two Cases and Review of Literature

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### Abstract

One case of suppurative pylephlebitis and another of solitary liver abscess of the left lobe without pylephlebitis following sigmoid diverticulitis are reported.

Forty-eight similar cases were collected. The pathologic and clinical features of 22, including the author's two cases, were reviewed. The primary focus in the sigmoid was silent in most of the cases. There were two types of pathologic presentation: 1) diverticulitis with pylephlebitis with or without multiple liver abscesses, and 2) diverticulitis with liver abscess without pylephlebitis. Incidence and diagnosis are discussed.

### Introduction

In 1906, Whyte (1) first reported a case of suppurative sigmoid diverticulitis with liver abscess. Kramer and Robinson (2) (1926) reported a similar case including pylephlebitis. Forty-six additional cases of liver abscess, with and without pylephlebitis, following diverticulitis have been collected from the literature (3–23). Because of the rarity of the complication of pylephlebitis and liver abscess following diverticulitis, and because of their obvious clinical importance, two additional cases are reported and the literature is reviewed.

### Case Reports

*Case 1.* This 69-year-old white woman (A68-44) was admitted to Mount Sinai Hospital Services, City Hospital Center at Elmhurst, on January 20, 1968, complaining of abdominal pain, loss of appetite, chills, and fever for 10 days. She had a radical mastectomy followed by cobalt therapy for carcinoma of the right breast at another hospital 2½ years previously. Six months after operation, severe pain occurred in the right hip, and the patient received radiotherapy. For the few months preceding this admission, she was receiving corticosteroids. Ten days prior to admission she developed fever and chills and had two episodes of nausea and vomiting. Tarry stools appeared for the past three days, and rectal bleeding was noted by her private doctor two days prior to admission. She developed jaundice and then became semicomatose on the day before admission.

On admission, the patient was semicomatose with shallow respiration and dehydration. Blood pressure was 80/50 mm Hg; pulse rate 110 per min; respiratory rate, 28 per min; temperature, 102 F. The skin showed jaundice with mild petechial hemorrhages. The abdomen was distended and showed generalized tenderness with rebound pain and rigidity. The liver was enlarged and tender. The spleen and kidneys were not palpable. Tarry stools were found during the rectal examination.

At the time of admission, white blood cell count was 5,000 per cu mm; hemoglobin, 14.8 g per 100 ml; and hematocrit, 49%. The urine was positive for bile. Blood urea nitrogen was 100 mg per 100 ml; serum alkaline phosphatase, 18 King-Armstrong units; uric acid, 25 mg per 100 ml; total bilirubin, 8.6 mg per 100 ml, with direct 6.5 mg per 100 ml; creatinine, 3.7 mg per 100 ml; serum glutamic pyruvic transaminase, 32 Sigma Frankel units; serum

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glutamic oxalacetic transaminase, 65 King-Armstrong units; lactic dehydrogenase, 150 Wroblewsky units; and aldolase, 8 Sibley-Lehninger units. The blood culture grew Proteus mirabilis, E. coli, and Aerobacter aerogenes which were sensitive to neomycin and Keflin®.

From the day of admission, the patient received neomycin 2 g four times a day by Levine tube and Keflin 2 g four times a day intravenously. Although the total bilirubin gradually decreased to 3.6 mg per 100 ml and alkaline phosphatase decreased to 14 King-Armstrong units, blood urea nitrogen steadily increased to 248 mg per 100 ml. Throughout her course, she was in a comatose state and had a temperature 101 F and moist rales over the lung bases. She died 1 week after admission. Clinical diagnosis was: 1) acute peritonitis due to perforation of viscus and 2) gastrointestinal bleeding secondary to corticosteroid therapy or hepatic failure due to liver metastasis.

At autopsy, focal fibrinous peritonitis was found in the left lower abdominal cavity. There was no ascites. Multiple abscesses were seen in the adipose tissue adjacent to the sigmoid and rectum. There were many diverticula in the sigmoid colon, some of which were filled with pus. One diverticulum was perforated with abscess formation. The remainder were impacted with fecal material and blood clots (Fig. 1). The remaining gastrointestinal tract was unremarkable. The mesocolon showed multiple areas of hemorrhage (Fig. 1) which, on microscopic examination, proved to be septic thrombi with perivascular hemorrhage in the small mesenteric veins. The inferior mesenteric vein was completely filled with greenish pus (Fig. 2) and its terminal branches showed foci of suppuration. The proximal segment of the splenic vein between the entry of the inferior mesenteric vein and the portal vein was occluded by a septic thrombus. The remaining segment of the splenic vein was patent and filled with blood. The distal part of the portal vein contained a mural thrombus. The intrahepatic radicles of the portal vein were patent but on microscopic examination showed thrombophlebitis. The liver weighed 1,600 g and no abscesses were found grossly or microscopically. The gallbladder was distended and had a normal mucosa. The extrahepatic biliary ducts and main intrahepatic biliary ducts were patent. The spleen weighed 80 g and was congested and soft. The superior mesenteric

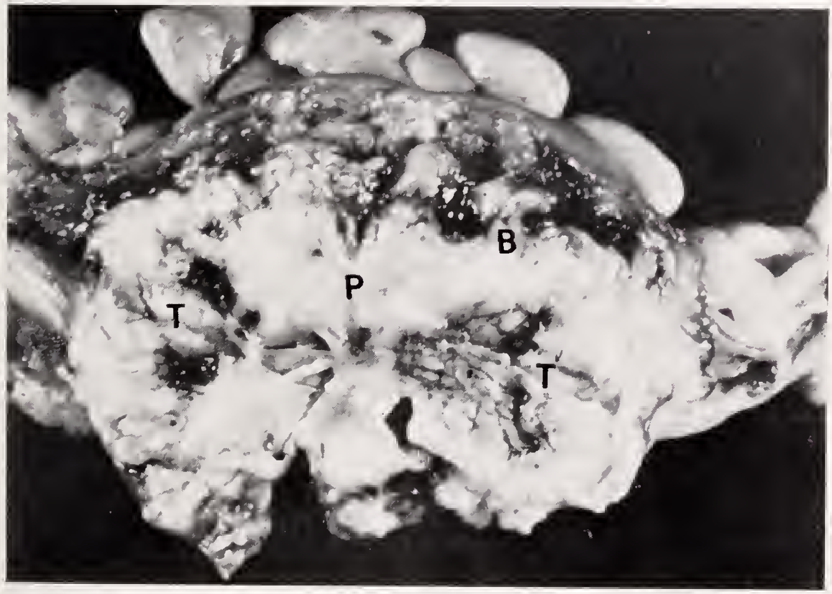

Fig. 1. Case 1. Cut section through the sigmoid colon and mesocolon showing acute diverticulitis with bleeding(B) and perforation(P), and septic thrombi(T) in the small tributaries of the inferior mesenteric vein.

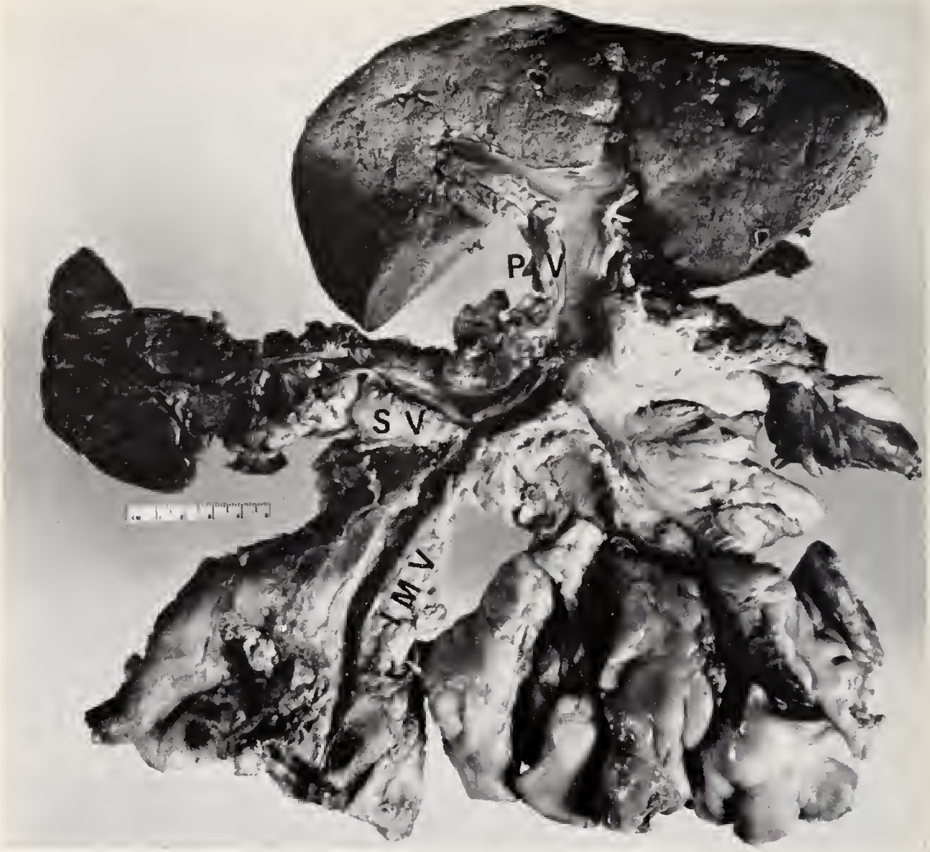

Fig. 2. Case 1. The portal venous system showing pus accumulation in the whole segment of the inferior mesenteric vein(IMV), an occluding thrombus with pus in the proximal part of the splenic vein(SV), and a mural thrombus in the distal part of the portal vein (PV).

vein and its tributaries were patent. Postmortem bacterial culture of pus grew Proteus mirabilis, Enterococci, E. coli, Pseudomonas aeruginosa, and Aerobacter aerogenes. No amoebae or fungi were found. Grossly, the liver was slightly icteric, and histologic sections showed bile plugs in a few bile canaliculi and intracellular cholestasis, especially around the central veins. The portal areas were markedly infiltrated with polymorphonuclear and mononuclear cells.

In addition, the autopsy disclosed metastatic carcinoma from the previously removed primary breast carcinoma in the skin of the right chest, lungs, adrenals, lymph nodes, and bones. A few microscopic metastatic foci were scattered in the liver.

*Case 2.* This 59-year-old white woman (A68-112) was admitted to Mount Sinai Hospital Services, City Hospital Center at Elmhurst, on February 28, 1968 complaining of anorexia, weakness, and shortness of breath of one-week duration. She had a history of diabetes mellitus for the past ten years and was taking Orinase®. Two and a half years ago, she had a fall with a fracture of her left lower leg with subsequent confinement to a wheelchair. At that time, she was found to have high blood sugar and was placed on insulin, 50 units per day. One week prior to admission, she began to feel weak and to lose her appetite and decided to discontinue insulin therapy. At the same time, she complained of shortness of breath and claimed that she could not lie flat in bed. She had a thyroidectomy many years ago and was allergic to penicillin.

On admission, she was obese, conscious, oriented, and in mild respiratory distress. The pulse rate was 132 per min; temperature, 100 F; respiratory rate, 26 per min; and blood pressure, 128/90 mm Hg. An operative scar was seen on the right side of neck. The lungs and heart were unremarkable. The abdomen was soft and nontender. The liver and spleen were not palpable. The rectal examination was unremarkable. A few shallow, small skin ulcers were present on the lower legs.

White blood cell count was 20,200 per cu mm, with 83 percent of polymorphonuclear cells. Sugar and acetone were present in the urine. Serum chloride was 89 mEq per liter; potassium, 3.5 mEq per liter; sodium, 131 mEq per liter; glucose, 544 mg per 100 ml; serum glutamic pyruvic transaminase, 91 Sigma Frankel units; serum glutamic oxalacetic transaminase, 220 King-Armstrong units; lactic dehydrogenase, 550 Wroblewsky units; total bilirubin, 0.7 mg per 100 ml with direct 0.4 mg per 100 ml; alkaline phosphatase, 20 King-Armstrong units; and blood urea nitrogen, 21 mg per 100 ml. The culture of skin ulcers grew Aerobacter aerogenes and Enterococci which were sensitive to Keflin and the Enterococci were also sensitive to erythromycin. The chest x-ray showed prominent vascular markings and blunting of the right costophrenic angle.

The patient was treated with regular insulin. A pulmonary embolism was suspected and she was placed on heparin initially and then Coumadin®. Erythromycin 500 mg four times a day was started for two days initially and then followed by 250 mg four times a day for two days. Because a sensitivity test of Aerobacter aerogenes to erythromycin was not done, she was switched to Keflin 2 g four times a day until death. She died on the sixth hospital day.

At autopsy, the left upper abdominal cavity was filled with 1,500 cc of purulent fluid and pus. Each pleural cavity contained 200 cc of serofibrinous fluid. The liver was markedly enlarged and weighed 3,000 g. There was a large solitary abscess in the dome of the left lobe of the liver (Fig. 3), and on serial cut sections, coalescent multiple abscesses were demonstrated. A few small perforations were seen on the surface over the abscesses. The portal vein and its main intrahepatic radicles were patent. Microscopically, a few small radicles of the portal vein adjacent to the abscesses were filled with fibrin thrombi. Most of the portal areas in the left lobe were markedly infiltrated with polymorphonuclear and mononuclear cells. The portal areas in the right lobe were also infiltrated with inflammatory cells, but to a lesser extent. A segment of a small branch of the hepatic vein connected to the abscess was filled with a blood clot which revealed thrombophlebitis microscopically. Bacterial culture of the abscess grew Proteus mirabilis, Enterococci, and E. coli. The splenic and mesenteric veins were patent and no pus or thrombi were seen in the vascular lumens. There were many diverticula in the colon from the ascending to the sigmoid portion. Most of the diverticula were impacted with fecal plugs and showed chronic inflammation with fibrotic thickening of the wall. A few diverticula in the sigmoid showed acute necrotizing inflammation with collection of pus in the lumens (Fig. 4). No amoebae or fungi were found in the liver abscess and in the acute inflammatory lesions of the diverticula. No inflammation was seen in the other abdominal visceral organs. The spleen weighed 280 g and was septic. The diaphragm showed acute inflammatory cell infiltration through muscle layer to the pleura. Both lower lobes of the lungs were atelectatic, especially the left lower lobe. The pancreas showed a mild degree of hyalinization in the islets of Langerhans. A mild degree of diffuse type of glomerulosclerosis was noted. Brain was normal.

## Review of Literature

Forty-eight cases of pylephlebitis and/or liver abscess following colonic diverticulitis were collected from the available literature (1–23). There were clinical features and pathological findings available in 20 cases (1–3, 7, 9–12,

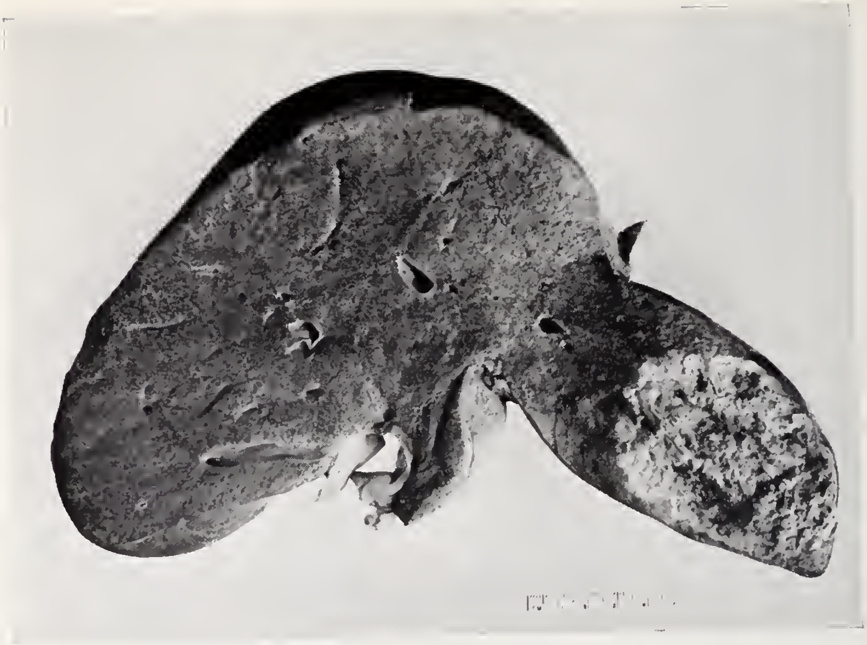

Fig. 3. Case 2. Section of the liver showing a large solitary abscess in the left lobe with perforations on the capsular surface.

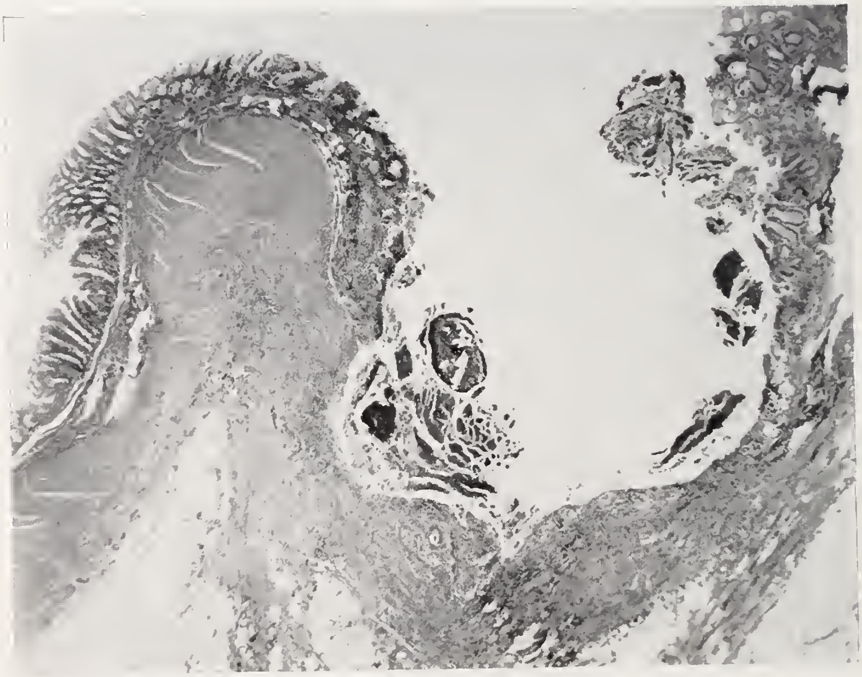

Fig. 4. Case 2. Photograph of the histologic section of a sigmoid diverticulum showing acute and chronic inflammation with pus and fecal plug in the diverticular lumen. (× 16)

14, 16, 21). Therefore, the review will be limited to these cases plus the two herein reported.

All the cases had combined acute and chronic diverticulitis or acute suppurative diverticulitis. Six cases were associated with intra- or paracolonic abscess and four cases with peritonitis. Four cases (2, 7, 10) had suppurative pylephlebitis involving to a variable degree the portal venous system from the inferior mesenteric vein to the intrahepatic radicles. Two of these four cases of pylephlebitis had multiple liver abscesses in both lobes and of the remaining two, one survived and the other died. Neither of these had a liver abscess. Of 17 of the 18 cases of liver abscess following diverticulitis that were reported, the status of the portal venous system including the splenic and inferior mesenteric veins were not adequately described. Multiple liver abscesses were seen in nine cases—four involving both lobes and five involving the right lobe. A solitary abscess was seen in seven cases—four involving the right lobe and three involving the left lobe. In the remaining two of these 18 cases of liver abscess no mention was made of the site and number. A subphrenic abscess was seen in 4 cases.

The patients' ages ranged from 34 to 77 and in more than 90 percent of the cases was above 50. The sex ratio of male to female was 3 to 1. The cardinal symptoms and signs were fever (99 percent), chills and rigors (64 percent), jaundice (60 percent), enlarged tender liver (55 percent), and leukocytosis (68 percent). Temperature was from low to high grade and was intermittent or persistent. Chills or rigors were not always associated with fever. Jaundice appeared initially or terminally and was transient or progressive. Jaundice and chills or rigors were invariably seen in four cases of suppurative pylephlebitis. Bowel symptoms and signs including constipation, diarrhea, loose stools and stools mixed with mucus, pus and blood were not common (32 percent) and could appear initially or terminally. Abdominal pain in the right upper quadrant (32 percent) or in the left lower quadrant (nine percent) were rare. Occasionally present were nausea, vomiting, sweating and chest symptoms and signs including shortness of breath, cough, rales and chest pain. Alkaline phosphatase was markedly increased in one case, slightly increased in two cases, and normal in two cases. Eleven cases had antemortem blood culture and only three cases grew Streptococcus viridans, E. coli, Proteus mirabilis and Aerobacter aerogenes. Three of four cases of suppurative pylephlebitis had positive blood culture. Bacterial culture from liver abscess was made in nine cases and eight cases grew E. coli, hemolytic Streptococci, anaerobic gram negative bacilli, Enterococci, and Proteus mirabilis.

Ten of the cases of liver abscess and/or pylephlebitis (10–12, 14, 16, 21) were diagnosed clinically by laparotomy or needle aspiration. Four (10–12) of these ten cases had an initial diagnosis of diverticulosis or diverticulitis and two of these four cases had bowel symptoms and pain in the left lower quadrant, and survived (10, 12). One case in Shaldon's series (11) had intermittent fever with chills and rigors for ten days and pylephlebitis was diagnosed on the 17th hospital day while enlarged tender liver with faint jaundice developed. This patient survived after drainage of liver abscess and ligation of the inferior mesenteric vein. From the available data, the clinical course ranged from 12 days to six months. The treatment included antibiotics, drainage of liver abscess (11, 12, 21), lobectomy (21), ligation (11), or excision (10) of the involved vein and excision of the primary infectious focus.

## Discussion

The major symptoms and signs of pylephlebitis and liver abscess following colonic diverticulitis were fever, chills and rigors, jaundice, enlarged tender liver, and leukocytosis. Symptoms and signs of bowel disorder were infrequent. Most of the cases had a silent primary focus as pointed out previously by Shaldon (11). The diagnosis was frequently difficult as reflected in the reported cases where a variety of incorrected clinical diagnosis was made. Lack of pain in the right upper quadrant and left lower quadrant were possibly because aged persons reacted minimally to the disease process (24). This might be the reason why so many cases including Case 2 in this report were misdiagnosed as conditions unrelated to liver abscess and colonic diverticulitis. Therefore, it is important to be aware of this kind of complication in patients over the fifth decade and to keep this complication in mind in the differential diagnosis of fever of unknown origin. The correct diagnosis can be established by laparotomy, percutaneous aspiration of pus and liver scan.

The incidence of suppurative pylephlebitis and liver abscess following colonic diverticulitis is rare. This may be attributed to the use of antibiotics and low virulence of E. coli and other gram negative bacilli, as well as efficient phagocytotic activity of the reticuloendothelial system of the liver. Acute diverticulitis with perforation during long-term corticosteroid therapy has been reported (25–27). Also reported was solitary pyogenic liver abscess in a patient with diabetes mellitus (28). Decrease of resistance to infection following corticosteroid therapy or uncontrolled diabetes mellitus may be precipitating factors in the two cases reported herein.

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# Liver Injury from Elixir of Terpin Hydrate with Codeine

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## Abstract

Hepatic injury with extensive fibrosis developed in a codeine addict. We believe that this liver disease was a complication of his codeine addiction, not resulting from the codeine itself, but rather from the elixir of terpin hydrate in the cough syrup he imbibed.

## Introduction

Some of the medical complications of narcotic abuse are not caused by the narcotic itself, but by the preparation in which the narcotic drug is given. For example, infected particulate material accompanying illicitly available heroin is responsible for the infectious complications of the heroin usage. Quinine used to dilute heroin has caused cardiotoxicity (1) and has been implicated as a possible cause of respiratory arrest associated with heroin overdosage (2). We have encountered another complication of narcotic abuse resulting, not from the narcotic itself, but from the vehicle in which the narcotic was taken. The following is a report of a codeine addict who developed cirrhosis from ingesting large quantities of elixir of terpin hydrate with codeine.

## Case Report

J.H., a 30-year-old-man, was admitted to City Hospital at Elmhurst because of a generalized convulsion. For the past five years he had been taking codeine in the form of 16 to 20 ounces of cough syrup each day. Two years ago he had a generalized seizure for the first time and was hospitalized at City Hospital Center. Lumbar puncture, brain scan, and electroencephalogram were normal. He did not have enlargement either of liver or spleen at that time, and results of liver function tests were all normal. He was given 300 mg diphenylhydantoin daily.

Following discharge he continued to imbibe up to 20 ounces of cough syrup each day. Two days prior to the present admission he developed a cough and fever. On the day of admission he had a generalized seizure.

He denied the parenteral self-administration of narcotics, previous blood transfusions, or previous episodes of hepatitis. He drank alcoholic beverages infrequently but he ate well every day. He worked as a delivery man and he stated that the people with whom he worked thought him to be peculiar because he was lethargic or inattentive.

The physical examination on admission revealed a moderately obese male in no acute distress and without jaundice. The temperature was 101.5 F; the pulse was 110 per minute. The blood pressure was 120/80 mm Hg. The neck veins were not distended. Bilateral gynecomastia was present. The lungs were clear, the heart tones were of good quality and there were no murmurs. The liver was palpated five fingerbreadths below the costal margin and it was slightly tender. The spleen was not felt. The testes were small. Ascites, peripheral edema and asterixis were absent. Neurologic examination was normal. The hemoglobin was 12.5 grams per 100 ml. The white blood cell count was 7,500/mm3. The platelets were 204,000/mm3. The erythrocyte sedimentation rate was 44mm/hr using the Westergren technique. Urinalysis was normal. No pathogens were found in blood, urine, and sputum cultures. The blood urea nitrogen was 3 mg per 100 ml. The serum protein was

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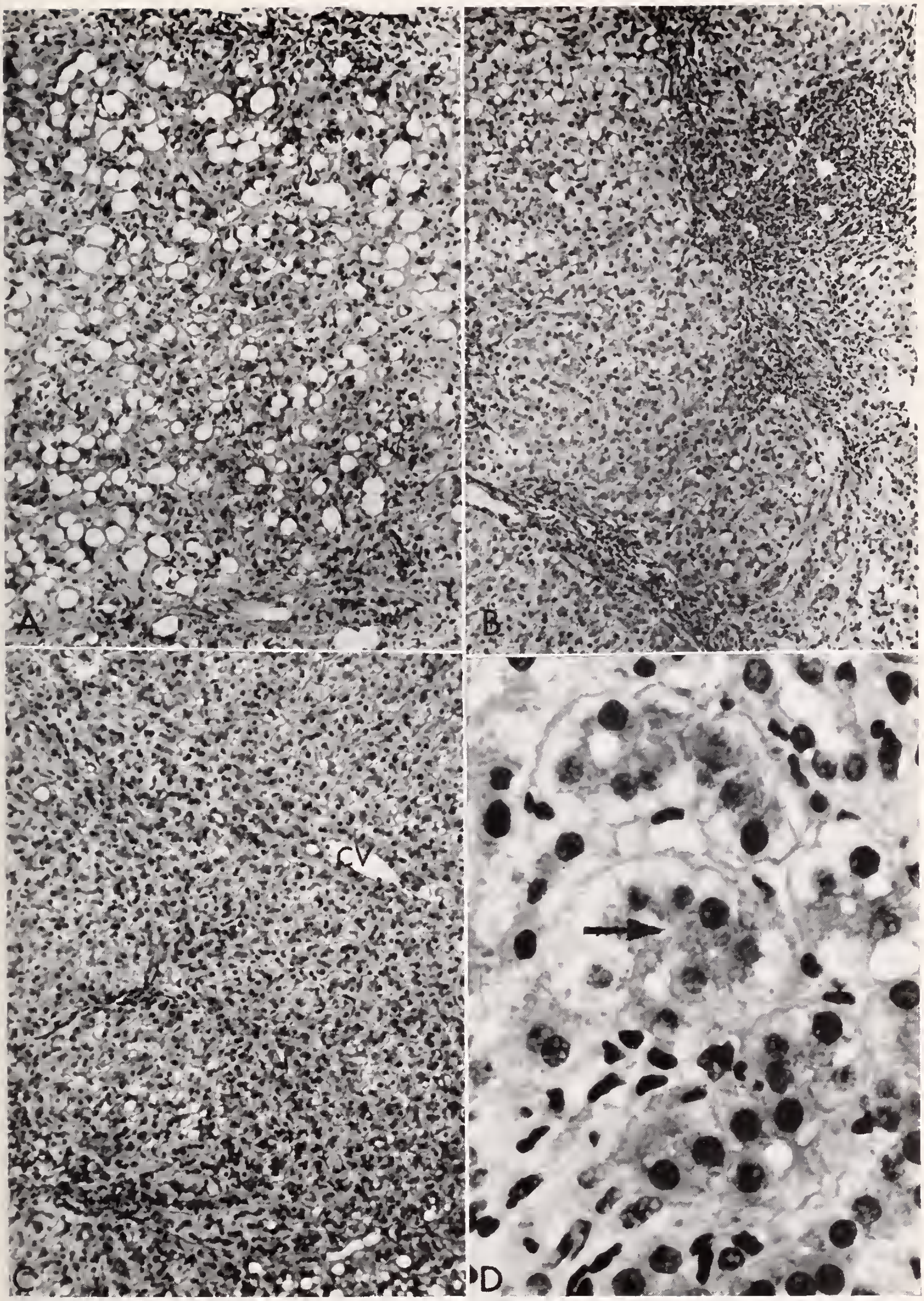

FIG. 1. Photomicrographs of the liver biopsy specimen (Hematoxylin and Eosin). a. Steatosis indicated by large round fat droplets (× 100). b. Inflammation and stellate fibrosis of portal tracts (× 100). c. Normal central vein (CV). Note the absence of fat in this lobule (× 100). d. Acinar formation of liver cells around a dilated bile canaliculus (× 630).

6.1 G/100 ml with 2.8 G/100 ml globulin. The serum bilirubin was 0.3 mg per 100 ml; alkaline phosphatase, 240 mili international units per ml; SGOT, 167 mu/ml; SGPT, 44 Karmen units per ml, LDH 221 mu/ml. The calcium and phosphorus were normal. A brain scan was normal. The liver scan showed an enlarged liver with patchy uptake and increased splenic uptake.

The patient was not given antibiotics. After two days of hospitalization his temperature returned to normal. A liver biopsy was performed which showed mild steatosis (Fig. 1a), which did not involve all lobules. The portal tracts were enlarged, stellate in shape and infiltrated with mononuclear cells (Fig. 1b). Piecemeal necrosis was seen in some of these portal areas. Bile ducts were proliferated with increased fibrous tissue around them and with fibrous septa connecting adjacent portal areas (perilobular fibrosis). Central veins were normal (Fig. 1c) and alcoholic hyaline of Mallory was not seen. Many plates of hepatocytes were two cells thick, and often hepatocytes showed an acinar arrangement around a dilated bile canaliculus (Fig. 1d).

## Discussion

According to the Bureau of Narcotics, about 25 percent of the codeine used in the United States is in the form of cough mixtures (3). It is in this form that codeine is most readily accessible to addicts. In addition to codeine, these cough syrups usually contain 39 to 44 percent of alcohol (4) and terpin hydrate.

The patient described has both clinical and laboratory evidence of hepatic dysfunction. His liver biopsy showed fat, inflammation, bile duct proliferation, and increased connective tissue with perilobular fibrosis. These changes could have been caused by alcohol. The 16 to 20 ounces of cough syrup he used each day, probably contained about 200 ml of pure ethyl alcohol. This amount of alcohol is sufficient to produce changes in the liver. This can include steatosis, alcoholic hepatitis, or even cirrhosis (5).

Evidence that codeine adversely affects the liver is minimal. Foulk and Fleisher (6) found elevated serum transaminase levels in man, following the acute administration of large doses of codeine. Others have not confirmed this (7, 8). Also, the rate of metabolism of hexabarbital by rat livers, appears to be diminished by codeine (9, 10). However, codeine by itself has not been reported to produce hepatitis or cirrhosis. Diphenylhydantoin has produced hepatic reactions, occasionally with formation of granulomas. Toxicologic studies with long-term administration of large doses of terpin hydrate are lacking. Since the lesion found did not resemble any of those usually associated with alcoholic liver disease (11), and since no granulomas were noted, the only agent which can be blamed for the liver disease is terpin hydrate. A role for alcohol in the potentiation of a toxic effect of terpin hydrate is possible since the effects of other poisons like carbon tetrachloride are enhanced by alcohol. Malnutrition does not seem to be a factor in this case although the cough syrup contributed about 1,000 calories a day to his intake.

Protracted use of agents producing hepatic injury may lead to chronic hepatitis (12). Whether terpin hydrate should be considered capable of causing chronic hepatitis cannot be answered in this case because immunologic studies were not performed.

### Acknowledgment

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# Fibromuscular Hyperplasia of the Extracranial Arteries: Report of a Case Associated with Intracranial Aneurysm and Skeletal Deformities and a Brief Review of the Literature

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Fibromuscular hyperplasia (FMH) is an arterial disease of unknown etiology. It was first described in 1938 by Leadbetter and Burkland (1) in a case with renal artery involvement. Little further appeared in the literature until 1960 when the relationship of renal artery FMH to hypertension was suggested (2). Subsequent reports bore out the relationship with surgically correctable hypertension (3–6). In 1964 the first case of extrarenal (coeliac artery) involvement was described (7), and Connett (8) in 1965 reported radiologic and histologic proof of involvement of the extracranial vessels. Since Connett's report several single case presentations (9–14) and a number of series (15–23) of FMH affecting the extracranial vessels have appeared. This paper will review the English language literature and report a case associated with skeletal deformities and intracranial aneurysm presenting as a subarachnoid hemorrhage. The purpose is to reassert the association of intracranial aneurysm with FMH and to suggest a possible underlying basis for the arterial abnormality.

## Case Report

A 43-year-old white woman was admitted to the Beth Israel Hospital in New York in February 1972 with progressive right-sided weakness and speech difficulty of 24 hours' duration. The symptoms began with an intense left parietal headache. There was no previous neurologic symptomatology.

Examination revealed a lethargic female with a B.P. of 140/80. A pectus excavatum and bilateral pes cavus deformities were present. Speech was limited to unintelligible monosyllables. The right pupil measured 4 mm, the left 2 mm; both reacted to light. A right homonymous hemianopia and right facial paresis were present. The remainder of the cranial nerves were normal. There was a right hemiplegia and a Babinski sign on the right. There was no gross sensory deficit. Signs of meningeal irritation were absent on admission but subsequently developed. A bruit over the right carotid artery was heard consistently. Transient bruits in the right supraclavicular fossa and over the left carotid were also heard.

Laboratory studies including a complete blood count, sedimentation rate, prothrombin time, and routine blood chemistries were normal. A lumbar puncture showed hemorrhagic CSF under normal pressure, total protein of 108 mg%, 432 RBC's, 2 WBC's, and 1+ xanthochromia. An EEG showed bilateral slowing most marked on the left. Angiographic studies included a direct, percutaneous left carotid angiogram, a right brachial angiogram, and an abdominal angiogram. These revealed changes as seen in FMH in both internal carotid and the right vertebral arteries, and an aneurysm of the left internal carotid artery at the level of the anterior clinoid processes (Figs. 1 and 2.). There was no involvement of the renal or extrarenal abdominal arteries. A scout film taken during the right brachial angiogram showed changes consistent with FMH in the right brachial artery (Fig. 3.).

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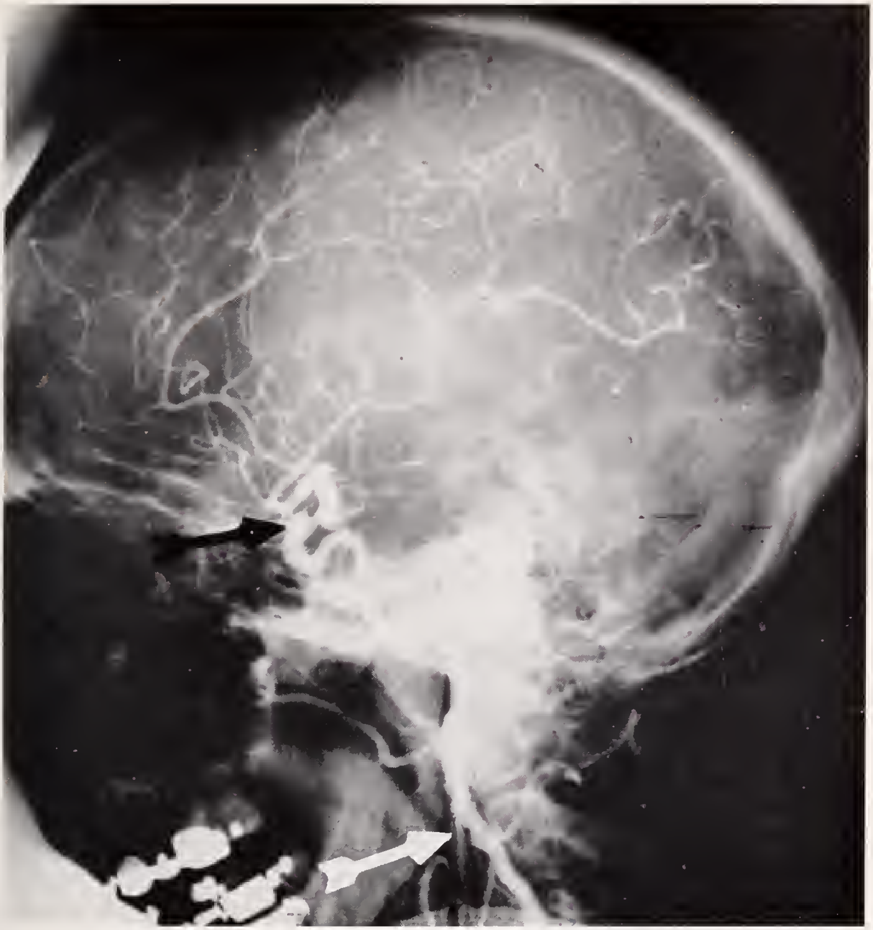

Fig. 1. Left internal carotid angiogram showing changes of FMH in the cervical portion of the left internal carotid artery (white arrow) and an aneurysm at the level of the anterior clinoid processes (black arrow).

Treatment consisted of bed rest, antihypertensive medications, and parenteral steroids. The course has been one of gradual but incomplete clearing of both the aphasia and the right hemiplegia.

Addendum: There is a family history of skeletal deformities. The patient's mother had bilateral pes cavus; neither of the patient's two siblings was affected. Of the patient's two children, one male has pectus excavatum. There is no family history of hypertension or neurologic dysfunction, however.

## Review of the Literature and Comment

### *Incidence and Etiology*

Since 1965, 91 cases (including this report) of FMH affecting the extracranial vessels have been described. The majority of these reflect the experience at the Mayo Clinic (44 cases) (22), Baylor (13 cases) (21), and University of Cali-

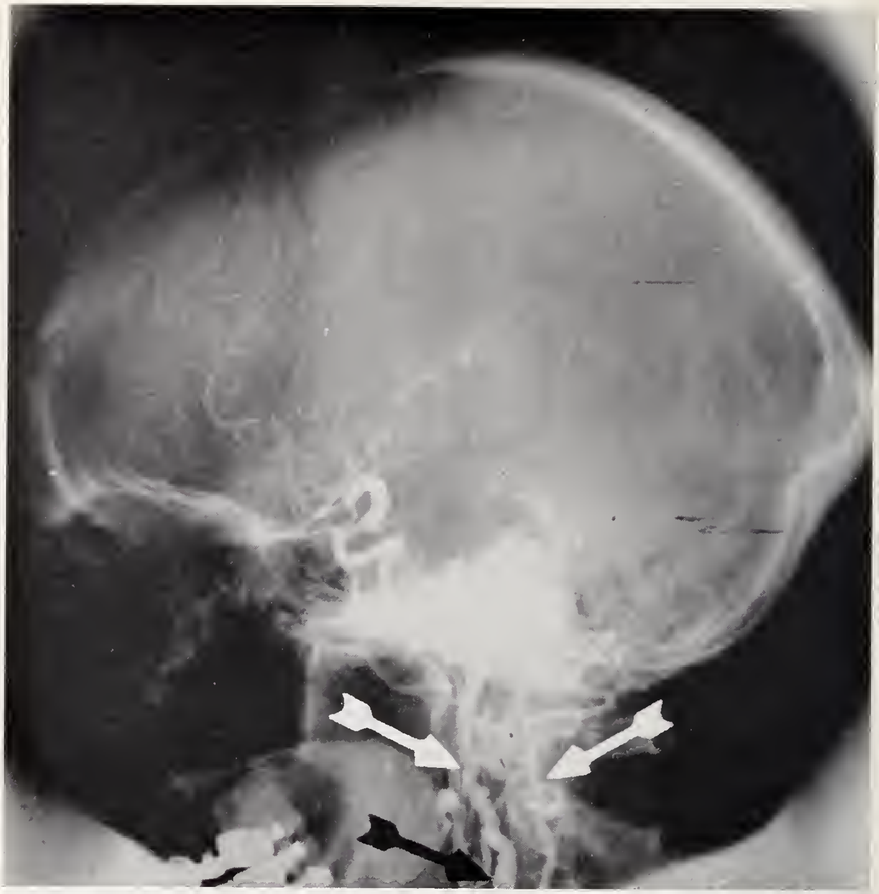

Fig. 2. Right brachial angiogram showing characteristic beading of both the right carotid and vertebral arteries (white arrows). Note the sparing of the proximal segment of the right internal carotid artery (black arrow).

fornia at San Francisco (6 cases) (19, 20). The exact incidence of the entity is unknown. In two large reviews of cerebral angiograms, FMH was discovered in 5/2000 = 0.25% (17) and 44/7000 = 0.63%. (22) All cases have been Caucasians; average age was 52 (range 8–82), 77/91 = 85% were female. No etiology is known. Halpert (24) reported renal artery involvement in three hypertensive sisters and suggested a genetic basis. Harrison (25) suggested several possibilities including abnormalities of the vasa vasorum and stress effects on the arterial wall. The presence of FMH predominantly in arteries receiving a high percentage of the cardiac output has been discussed in relation to a possible etiology (17). An endocrinologic basis has been postulated because of the higher incidence in females than males (10). Andersen (26) suggested an inflammatory basis.

Our case has associated skeletal abnormalities, namely bilateral pes cavus and pectus excavatum, with a positive family history. No previous reports of

FMH and such defects could be found; however, from an etiologic standpoint our case leads us to draw the analogy of FMH to Marfan's syndrome. The latter, a hereditary disorder of connective tissue, is associated with diffuse and/or dissecting aneurysms of the ascending aorta and skeletal abnormalities. Pectus

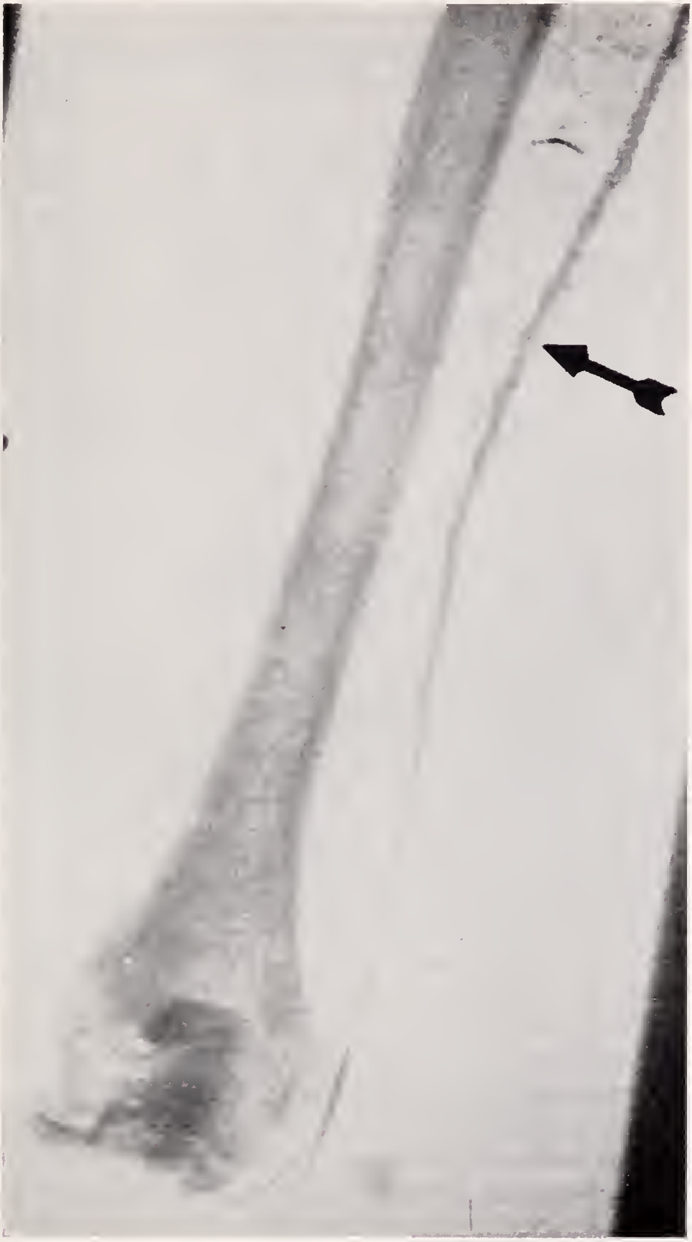

Fig. 3. Scout film of the right brachial artery: Changes consistent with FMH are seen (black arrow).

excavatum, a reflection of excessive growth of the ribs, is a frequent associate. Pes cavus has also been described but less frequently than pes planus (27). Aneurysms in association with Marfan's syndrome usually occur in the ascending aorta. Hardin (28) reported a case with an aneurysm of the extracranial portion of the internal carotid artery. (The possibility of coincidental occurrence is not to be disregarded.) The pathologic changes in the arterial wall in advanced cases are of note. They consist of fragmentation and sparsity of elastic tissue, hypertrophy of smooth muscle, increase in fibrous elements, dilatation of the vasa vasorum, and cystic spaces filled by metachromatically staining material (27). These changes are similar though not identical to those found in arteries involved by fibromuscular hyperplasia. The similarity between the clinical and pathologic abnormalities in our patient with FMH and those found in Marfan's syndrome suggests a possible common etiologic basis. We wish to suggest that FMH may be a reflection of an hereditary, diffuse, mesenchymal disorder rather than a specific arterial defect.

### *Symptomatology*

Only four of 91 cases presented without neurologic complaints. Of the remainder, 30 had symptoms of transient cerebral ischemia referable to the cerebral distribution of the artery (ies) shown to have FMH by angiography (12, 14, 18–23). The remaining 57 cases were discovered unexpectedly during the investigation of presumably unrelated neurologic conditions such as seizures (psychomotor (15), grand mal (9, 10), and focal (23)), atherosclerosis of the extracranial vessels (13, 14, 19), subdural hematoma (16), head injury without subdural hematoma (16, 22), intracerebral neoplasm (16, 22), arteriovenous malformation (10), headache syndromes including migraine (15, 22, 23), demyelinating disease (22), and tic douloureux (22). The incidence of FMH in each of these is so low as to suggest nothing more than chance association. There is gathering evidence, however, to support a more than coincidental occurrence of intracranial aneurysm with FMH of the extracranial vessels. Wylie (9) in 1966 reported 70 cases of renal artery FMH. Of these, six had involvement of either one or both carotid arteries, and five of the six had intracranial aneurysms. Eighteen additional such cases (including ours) have since appeared in the literature (15, 21–23)—an incidence of 23 of 91 (25 percent). This far exceeds the accepted incidence of aneurysms of two to five percent (29) in the general population. Whether this indicates a causal relationship between the FMH and aneurysms in such cases or a second, more basic etiology underlying both defects has yet to be determined. That some relationship exists seems indisputable. Also of note is the report of two patients with FMH confined to the intra-abdominal vessels who suffered subarachnoid hemorrhages (30). At postmortem neither showed evidence of intracranial aneurysm nor changes of FMH in the intracranial vessels. Possibly these cases represent a transitional phase, lying between normal vessel wall and aneurysm formation, in the pathology of FMH.

### *Radiologic Findings*

Angiographically FMH appears as alternating zones of constriction and dilatation of the arterial lumen, the so-called "string of pearls" or "beaded vessel" effect. The dilatations are always wider than the normal arterial lumen and the proximal 2.0 to 2.5 cm of the internal carotid artery is usually spared (31). The process rarely extends beyond the carotid canal, though cases with intracranial involvement have been reported (15, 23). Other arterial defects may present a similar angiographic picture. Stationary arterial waves of the carotid arteries may occur in cases with slowed intracranial circulation. These give a regular, saw-toothed appearance causing narrowing of the midportion of the vessels. Belber (32) reported a case of FMH involving the renal arteries and associated with intracranial aneurysm in which stationary waves in the left internal carotid artery mimicked the changes of true FMH. Circular spastic contractions appear as a uniform narrowing of the internal carotid, the proximal 2.5 cm is not spared, and the dilatations are not wider than the normal lumen (31). The internal carotids are the extracranial vessels most frequently affected, numbering 85 of 91 cases (93 percent). The vertebral arteries were involved in 13 of 91 (14 percent). One report of external carotid and superficial temporal artery involvement has appeared (15).

### *Pathologic Findings*

FMH affects small- and medium-sized arteries. It may be localized to one area or be multifocal with affected areas separated by a normal appearing vessel wall (22). The involved arteries have areas of medial thickening secondary to hypertrophy of smooth muscle and fibrous elements. Areas adjacent to such regions consist of thinned intima, disruption of the elastic membrane, and a markedly thinned media composed predominantly of fibrous tissue (17). Crocker (33) divided the pathology of FMH into two classes affecting predominantly the media and adventitia respectively. She postulated disruption of the arterial elastica as the primary defect in FMH and suggested that the areas of medial thickening reflect compensatory muscular hypertrophy. These pathologic changes correlate well with the roentgenographic appearance of a "beaded" vessel. The similarity between the histology of FMH and that of intracranial aneurysm has been discussed (19) and is another observation to support the relationship of the two conditions.

### *Treatment*

Medical treatment has consisted in the use of anticoagulants in cases with symptoms of transient cerebral ischemia. Only four such cases have been reported. Symptoms were alleviated in all four with follow up for 18 months (18, 22). Various surgical procedures have been used including saphenous vein grafts (8, 17), patch graft angioplasty (13), and resection and reanastomosis (12). Morris, Lechter, and DeBakey (21) instituted a procedure of progressive dilatation of the arterial lumen using graduated bile duct catheters. Using this procedure alone or in association with resection or patch graft angioplasty, they reported alleviation of symptoms in six patients with a follow-up of two months to four years.

## Summary

A case of fibromuscular hyperplasia (FMH) involving both internal carotid arteries, the right vertebral and the right brachial arteries associated with skeletal deformities and an aneurysm of the left internal carotid artery has been presented. A review of the literature has revealed 91 cases of FMH affecting the extracranial vessels. Only 33 percent of cases present with symptoms directly related to the process. Twenty-five percent of cases have been associated with intracranial aneurysms. The various other clinical, radiologic, and pathologic aspects of FMH have been reviewed. A possible association of FMH with a more diffuse mesenchymal tissue disorder is suggested.

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# Appearance of Nelson's Syndrome despite Pituitary Irradiation prior to Bilateral Adrenalectomy for Cushing's Syndrome

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To date there is no completely satisfactory treatment for pituitary-dependent bilateral adrenal hyperfunction, or Cushing's disease (1, 2). Total bilateral adrenalectomy offers a rapid and dependable cure (1–4), but a serious complication has been the development of pituitary tumors in about 10% of the patients (5–8). Characteristically, these tumors are chromophobe adenomas which grow rapidly and often require hypophysectomy (8–10). This complication, sometimes called Nelson's syndrome, is accompanied by hyperpigmentation, although some patients develop hyperpigmentation in the absence of a clinically demonstrable tumor (5, 11).

An alternative form of therapy for Cushing's disease is conventional external irradiation to the pituitary which, in doses of 4000–5000 rads, has induced clinically acceptable remissions in 30–50 percent of patients (4, 8, 9, 12). While the cure rate is limited and the remissions are delayed, this technique has the advantage of being noninvasive, readily available, and having few complications. Consequently, irradiation to the pituitary has been advocated as the initial mode of therapy in Cushing's disease and can be followed by adrenalectomy if remission does not ensue (1, 2, 4, 7, 12). Orth and Liddle (4) recently treated 19 patients with Cushing's disease by pituitary irradiation prior to total adrenalectomy. In none of these patients did a pituitary tumor develop during a follow-up period of one to thirteen years. As the tumor of Nelson's syndrome arises on the average three years after total adrenalectomy, the possibility has been raised that prior irradiation to the pituitary may prevent Nelson's syndrome (1, 2, 4). We wish to report a patient with Cushing's disease in whom pituitary irradiation did not prevent the development of a pituitary tumor.

## Case Report

S. W., a 47-year-old white male, was admitted to The Mount Sinai Hospital in April 1965, with a one-year history of rounding and redness of the face, polyuria, nocturia, and irritability. For six months he had experienced recurrent skin infections, decreased libido, easy bruisability, and leg weakness.

Physical examination revealed centripetal obesity, Cushingoid facies, buffalo hump, and purple abdominal striae. The skin was normally pigmented, with moderate acne. The blood pressure was 150/115 mm Hg.

Laboratory data included: hemoglobin 12.7 grams and white blood cell count, 8800/mm$^3$, with 84% neutrophils. Serum sodium was 153mEq/L; potassium, 3.3mEq/L; chloride, 113mEq/L; and bicarbonate, 34mEq/L. Blood urea nitrogen was 33 mg%. The oral glucose tolerance test was diabetic. The results of endocrine tests are shown in Table I. Baseline

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TABLE I

| | Baseline | Dexamethasone 2 mg/d × 2d. | | Dexamethasone 8 mg/d × 2d. | | ACTH 40 units |
|---|---|---|---|---|---|---|
| Urine 17 KS | 30.0 mg/24h | 17.4 | | 19.2 | | 30.7 |
| Urine 17 OHCS | 44.2 mg/24h | 27.3 | | 25.1 | | 89.7 |
| Plasma 17 OHCS | | | | | | 45.5 → 120 mcg% in 2h |
| | Fasting | ½hr. | 1hr. | 2hr. | 1hr. | |
| Glucose mg% | 118 | 226 | 318 | 224 | 120 | |

urinary titers of the neutral 17-ketosteroids and of the 17-hydroxycorticoids were elevated and were partially suppressed with 8 mg of dexamethasone a day. Roentgenograms showed a normal sella turcica and osteoporosis of the lumbar spine.

The patient was treated for three months with SK&F 12185® (dl-2(p-Aminophenyl)-2-phenyethylamine), an 11$\beta$-hydroxylase inhibitor (13, 14). Facial and abdominal fullness decreased moderately, the face and abdominal striae became paler, and the libido and the glucose intolerance improved significantly. In August of 1965, the left adrenal (17 grams) was removed and external irradiation (4500 rads) was given to the pituitary. The patient remained floridly Cushingoid with a high cortisol secretion rate of 60 mg per day. A second course of SK&F 12185® failed to suppress the hypercortisolism and the right adrenal was removed in April of 1968. The patient was placed on cortisone acetate 37.5 mg a day and 9$\alpha$-fluorohydrocortisone 0.1 mg per day. All manifestations of Cushing's disease promptly disappeared. About three years later, increasing skin pigmentation was first noted. In January, 1972, he was found to have a right inferior homonymous hemianopsia and roentgenographic evidence of an enlarged sella turcica with destruction of the floor. The serum protein-bound iodine titer was normal and carotid angiography was negative. Hypophysectomy was advised. Over the next few months the patient gained 20 pounds and his libido again became markedly depressed. In April, 1972, he was awakened at night by the sudden onset of severe, continuous, retrobulbar and occipital headache with vomiting. On admission he was found to have developed a larger deficit in the right homonymous field as well as a new deficit in the left homonymous field. Roentgenograms showed further sellar erosion with a soft tissue mass invading the sphenoid sinus. Carotid angiography now showed suprasellar extension. The cerebrospinal fluid was normal except for a protein of 73 mg%. The 24-hour $^{131}I$ uptake was 4% and the serum protein bound iodine level 3.0 mcg%. The patient was noted to have polyuria of about 8000 cc per day, polydipsia, and persistent, mild hyponatremia. He was receiving dexamethasone 3 mg three times a day, 9a-fluorohydrocortisone 0.2 mg daily, and sodium chloride tablets. No evidence of ADH deficiency was demonstrated by the diagnostic dehydration procedure of Miller and Moses (15). It was concluded that the polyuria was secondary to polydipsia, possibly due to hypothalamic damage. The patient was transferred to the neurosurgical service for management.

## Comment

In review of the literature, we have found no other case of Nelson's syndrome arising where the pituitary had been irradiated prior to total adrenalectomy. The only exception is a brief reference to such an occurrence made by Christy (9).

Our patient was initially treated by unilateral adrenalectomy in association with pituitary irradiation. The remaining adrenal was removed two and one-half years later when it became apparent that the initial form of treatment had failed. It is conceivable that removal of the first adrenal may have stimulated the development of a pituitary tumor before the suppressive effects of radiotherapy had taken effect. Pituitary tumors have been reported following subtotal adrenalectomy, when no pituitary irradiation has been given (16). However, we do not believe this was the case in our patient since no significant hypocortisolism was achieved to stimulate tumor development. It is of note that three of Orth and Liddle's patients followed the same therapeutic course as our patient without developing pituitary tumors (14). This, and another reported series (12), support the view that unilateral adrenalectomy is not a stimulus to pituitary tumor development. It is also clear that the persistent hypercorticism, which not infrequently follows pituitary irradiation, unilateral adrenalectomy, or a combination of both, does not indicate the presence of pituitary tumor (4, 12).

Although the experience to date indicates that pituitary irradiation may reduce the incidence of Nelson's syndrome (4), its effectiveness has not been precisely established. For this reason, failures of such therapy should be reported. This may also clarify whether there is a period of time in the course of the treatment of Cushing's disease when pituitary irradiation is most effective.

## Addendum

Since the submission of this report, a similar occurrence in a 23-year-old male was observed and followed by Dr. Dorothy Krieger in the Endocrine Clinic of The Mount Sinai Hospital. This patient had undergone unilateral adrenalectomy elsewhere in 1961 at the age of 12 years. In 1962 conventional radiotherapy had been given to the pituitary. No remission occurred and the patient underwent total adrenalectomy in the spring of 1971. X-rays of the sella turcica and the visual fields had been normal on repeated occasions. In September 1972 the patient first noted increased skin pigmentation. In October 1972, clear-cut enlargement of the sella turcica with erosion of its floor was present on x-rays.

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# A Clinical Study of Intracranial Aneurysms

## A Preliminary Study of 59 Patients Who Died during Their First Admission to Mount Sinai Hospital because of Ruptured Intracranial Aneurysm, Conservatively Treated

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Subarachnoid intracranial hemorrhage is a critical threat to life. Clinical studies have shown mortality rates of 33 percent to 64 percent and the literature is replete with contradictory data and opinions regarding the prognosis, natural history, and therapy of the two major categories of intracranial subarachnoid hemorrhage, namely, those secondary to intracranial aneurysms and those to arteriovenous malformations (1–18).

Patients admitted to The Mount Sinai Hospital from 1952 to 1968 have been surveyed. The clinical experience at this institution is unique in two respects: there is a large neurological service which has a strong leaning to conservative management and, secondly, one of the members of the neurological staff, Dr. Paul Slosberg, has intensively pioneered a hypotensive technique in the treatment of acute intracranial subarachnoid hemorrhage due to ruptured aneurysm (19–21).

The patients whose clinical course will be analyzed were treated by conservative (i.e., supportive) measures and surgery (Table I). All patients treated acutely by Dr. Slosberg have been and are being reported separately by him.

One of the current confusing aspects in management of ruptured intracranial aneurysm is related to contrasting mortality and morbidity rates between conservatively treated and surgically treated patients. Many factors are involved—the location of the aneurysm on the arterial tree, its location with respect to the dominant hemisphere, the size of the aneurysm, the presence of multiple aneurysms, and the clinical state of the patient. Neurosurgeons vary as to their recommendations with respect to intracranial surgery or extracranial carotid artery ligation. There is a recent tendency by some neurosurgeons (22) to wait until the acute bleeding has abated. Mullan and Dawley (23) have advocated the use of 6-aminocaproic acid, an antifibrinolytic agent, to help the patient over the acute episode.

This paper comprises an assessment of all cases of ruptured intracranial aneurysms admitted to The Mount Sinai Hospital for the first time who died under conservative management. The study comprises 59 patients, all of whom had intracranial aneurysms documented by cerebral angiography, postmortem examination or both.

The age range of patients in the survey was 15–71 years of age, 22 of whom were male, 37 of whom were female (Table II). Thirty-five patients, or 59

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TABLE I

*Mode of Therapy*

| | Conservative | Surgical | Total |
|---|---|---|---|
| 1. Intracranial Aneurysm | | | |
| A. Ruptured | 117 | 90 | 207 |
| B. Unruptured | 54 | 17 | 71 |
| | | | 278 |

TABLE II

*Age and Sex Distribution*

| Age | Male | Female | Total |
|---|---|---|---|
| 0–19 | 1 | 0 | 1 |
| 20–29 | 2 | 2 | 4 |
| 30–39 | 4 | 3 | 7 |
| 40–49 | 5 | 12 | 17 |
| 50–59 | 5 | 12 | 17 |
| 60–69 | 4 | 7 | 11 |
| 70–over | 1 | 1 | 2 |
| | 22 | 37 | 59 |

percent were between the ages of 40 and 60. This correlates with the National Cooperative Study in which 52 percent fell into this age group. In that study the male to female ratio was approximately two to three. The decades of highest incidence among our patients were 40–49 and 50–59 (17 patients each).

The most common aneurysm in this group of patients was the anterior communicating aneurysm (Table III). The frequency of the aneurysms at various sites in our series was approximately the same as those for other large series. Those patients with multiple aneurysms did not seem to have any more fulminating course than those with single aneurysms.

The frequency of initial symptoms is listed in Table IV. This agrees well with other such tabulations in similar patients. The most common symptom constellation was that of sudden headache accompanied by a change in mental status.

Since it is well known that acute intracranial bleeding can cause an elevation of systemic blood pressure, the observation of hypertension on admission was not considered evidence of the presence of this systemic illness. However, 19 of our patients had a history of hypertension before the onset of central nervous system symptomatology. One of these patients developed subarachnoid hemorrhage during a hospitalization for other complications of malignant hypertension.

It is noteworthy that 22 percent of the patients admitted to the hospital died within the first 24 hours. More than 50 percent died within the first five days.

## TABLE III

*Location of Intracranial Aneurysms*

| | |
|---|---|
| Internal Carotid A. | 11* |
| Middle Cerebral A. | 15** |
| Anterior Cerebral A. | 9 |
| Posterior Cerebral A. | 0 |
| Anterior Communicating A. | 20 |
| Posterior Communicating A. | 14*** |
| Basilar A. | 4 |
| | — |
| | 73 |

* Three patients had bilateral internal carotid artery aneurysm.

** Two patients had bilateral middle cerebral artery aneurysm.

*** Two patients each had a middle cerebral and an anterior cerebral artery aneurysm.

## TABLE IV

*Symptoms Leading to, or Present at, Admission to Hospital*

| | |
|---|---|
| Eye pain | 1 |
| Double vision | 2 |
| Lid droop | 2 |
| Headache | 33 |
| Difficulty in talking | 1 |
| Confusion | 24 |
| Neck pain | 4 |
| Weakness of arm and/or leg | 4 |
| Severe lethargy | 7 |
| Convulsion | 4 |
| Nausea and/or vomiting | 4 |
| Vertigo (felt "spinning") | 1 |
| Loss of vision of one or both eyes | 1 |
| Unsteadiness | 2 |
| Coma | 23 |
| Transient loss of consciousness | 6 |

Of those who died within the first 24 hours, eleven of fourteen had an onset of symptoms on the day of admission and of these eleven, nine developed coma soon after developing early symptoms. In the total group of patients, twenty-two developed coma on the first day of illness and all except three of these were hospitalized at Mount Sinai on that particular day. Of the patients who died within the first week, seventeen of thirty-eight were comatose on admission. Of those dying after the first week, five of twenty-four were comatose on admission.

Transient loss of consciousness does not seem to carry as poor prognosis as coma. Six patients had transient loss of consciousness as one of their presenting complaints. None of these died on the day of admission. Five of the fourteen patients dying on the first day had anterior communicating artery aneurysms

TABLE V

*Clinical State at Time of Admission*

| Stage | |
|---|---|
| I | 2 |
| II | 2 |
| III | 10 |
| IV | 45 |

Of significance is the large preponderance of patients in this series in the last two categories.

but this is also the most frequent site of intracranial aneurysms (30 percent in the National Survey).

The patients were clinically grouped as follows:

| | |
|---|---|
| Stage I | Neurological signs and/or symptoms without evidence of meningeal irritation. |
| Stage II | Meningeal irritation without other neurological signs or symptoms. |
| Stage III | Meningeal irritation and mild signs or symptoms, i.e., double vision, cranial nerve abnormalities, mild hemiparesis. |
| Stage IV | Meningeal irritation and moderate or severe signs or symptoms, i.e., organic mental syndrome, lethargy, aphasia, papilledema, moderately severe hemiparesis. |

The grouping—Stage I to IV—parallels the Cooperative Study grading system, Grades I to V. A "comatose" Stage IV patient would be Grade V (Table VI).

According to the recent thinking of some neurosurgeons only four of these patients would have been considered a good risk for surgery. A brief summary of each case follows.

## Case Reports

*Case No. 1.* A 27-year-old woman developed sudden severe head pain two weeks prior to admission. At the time of hospitalization she suffered a grand mal convulsion. On admission she had an organic mental syndrome, a right hemiparesis, a right Babinski sign, and nuchal rigidity. She stabilized and was essentially normal about the eleventh hospital day. Lumbar puncture revealed xanthochromia. On the seventeenth hospital day she lapsed into coma and died. Postmortem examination revealed a ruptured anterior cerebral aneurysm at its junction with the internal carotid artery.

*Case No. 2.* A 29-year-old hypertensive man developed sudden severe headaches one week prior to admission. Neurological examination on admission was normal except for papilledema and nuchal rigidity. Lumbar puncture revealed cloudy xanthochromic fluid, initial pressure 220 mm; 7150 rbc, 95% fresh; 330 wbc; 180 polycytes; 150 lymphocytes; and protein 336 mgm percent. The patient's clinical state improved and was stabilized until the 9th hospital day when he suddenly became comatose and died. Postmortem examination revealed a ruptured left anterior cerebral artery aneurysm.

*Case No. 3.* A 23-year-old woman developed a sudden severe eye pain, double vision and drooping of the right eyelid one week prior to admission. Neurological examination on ad-

## TABLE VI
*Clinical Summaries*

| Case # | Sex | Age | Stage | Angiography | Aneurysm | Summary |
|---|---|---|---|---|---|---|
| 5 | F | 60 | III | LC | L PCo | Six days prior to admission, sudden severe head pain. 10 hours prior to admission inability to express herself. On admission semicomatose with aphasia. Patient's neurological state was unchanged throughout 4 weeks. She suddenly developed a convulsion and died. Postmortem—ruptured posterior communicating artery aneurysm |
| 6 | F | 55 | IV | LV LC RC | ACo | Hypertensive. One day prior to admission sudden onset of confusion and head pain. On admission organic mental syndrome and nuchal rigidity. On 3rd hospital day she became more stuporous and lumbar puncture revealed additional hemorrhage. Progressive downhill course with death on 19th hospital day. |
| 7 | M | 44 | IV | LC RC | ACo | Nine days prior to admission sudden onset with progression of head pain. On day of admission found comatose. Emergency carotid arteriorgraphy revealed a right frontal lobe mass. Died within 24 hrs. Postmortem—ruptured ACo artery with a right frontal intracerebral hematoma. |
| 8 | F | 69 | III | RC IC | RMCe<br>LMCe | One day prior to admission sudden onset of severe head pain and stiff neck. On admission organic mental syndrome, left hemiparesis and stiff neck. Right carotid arteriogram revealed spasm of the anterior and middle cerebral arteries, and an IC aneurysm and two aneurysms of the middle cerebral artery. Patient gradually lapsed into coma and died on the 14th hospital day. |
| 9 | M | 32 | IV | None | PCo | One week prior to admission sudden onset of coma. Admitted to another hospital initially. On admission decerebrate state existed. Progressive deterioration with development of gastric hemorrhages. Died 3 days after admission. Postmortem—ruptured PCo artery aneurysm. Stomach demonstrated multiple small erosions. |
| 10 | F | 47 | IV | None | IC | Sudden onset of coma on day of admission. Remained critically ill and died on the 3rd hospital day. Postmortem—ruptured aneurysm, internal IC. A similar unruptured aneurysm was noted on the other side. |
| 11 | F | 51 | IV | RC | IC | Hypertensive. Sudden onset of severe confused state and left hemiplegia. Progressive deterioration. Death on 7th hospital day. Postmortem—ruptured IC aneurysm. |
| 12 | M | 61 | IV | None | PCo | On day of admission sudden onset of headache and semicoma. Rapid downhill course and died on 8th hospital day. Postmortem—ruptured PCo aneurysm. |
| 13 | F | 47 | IV | RB | PCo | Hypertensive. 3 days prior to admission sudden onset of organic mental syndrome, papilledema, vertical nystagmus on upward gaze and nuchal rigidity. Progressive deterioration with recurrent hemorrhage on 5th hospital day. Died at that time. |
| 14 | F | 48 | IV | RC LC | LPCo, R IC<br>L LC | Eleven days prior to admission sudden onset of head pain with confusion. On admission organic mental syndrome, papilledema and nuchal rigidity. On 2nd hospital day and right 3rd nerve palsy developed. On 7th hospital day she had a left hemiparesis. Progressive downhill course and death 2 days later. |
| 15 | F | 50 | IV | RC LC | PCo | On day of admission sudden onset of headpain followed by semistupor with left-sided weakness. On admission semicomatose, left hemiparesis, bruit heard over left eye. Progressive downhill course with death on the 9th hospital day. Postmortem—ruptured posterior communicating artery aneurysm. |
| 16 | M | 41 | IV | None | ACo | Sudden onset on day of admission of unconsciousness. Within 5 hours comatose and right hemiplegia. On admission no change in neurological status. Died within four hours. Postmortem—ruptured ACo. |

TABLE VI—*Continued*

| Case # | Sex | Age | Stage | Angiography | Aneurysm | Summary |
|---|---|---|---|---|---|---|
| 17 | M | 31 | IV | RC | PCo | Two hours prior to admission sudden onset of headache followed by unconsciousness. On admission semicomatose, bilateral Babinskis, nuchal rigidity. Progressive downhill course, developed gastric bleeding and died on 13th hospital day. |
| 18 | F | 47 | II | RC | ACe | Sudden head pain and stiff neck ten hours before admission. Right facial weakness and stiff neck. Bloody spinal fluid. Recurrent hemorrhage 5th hosp. day and again 7th day when she died. Postmortem denied. |
| 19 | F | 66 | IV | RB LC | RIC (cavernous portion)<br>RIC (at bifurcation) | One day prior to admission sudden onset of head pain with transient unconsciousness. On admission organic mental syndrome, nuchal rigidity. Progressive downhill course. Recurrent hemorrhage 6th day of admission with death the following day. |
| 20 | F | 48 | III | ? | ACo | Long history severe hypertension, sudden onset of severe headache, loss of consciousness 3 weeks prior to admission. Admitted elsewhere where LP bloody and angiogram performed. On admission semicomatose. During management of electrolyte and renal abnormalities (initial BUN 84) patient developed UGI bleed. 20 days after admission patient became more lethargic without evidence of recurrent bleeding. Died three weeks after admission. |
| 21 | M | 70 | IV | RC LC | ACo | Sudden onset of coma. Progressive deterioration with death on the 28th day of hospitalization. Postmortem—ruptured aneurysms ACo (not visualized by angiography). |
| 22 | F | 36 | IV | None | B | Hypertensive. Two weeks prior to admission sudden onset of headache and confusion. Followed by transient unconsciousness. One week prior to admission noted to have "disconjugate eye movements." On admission in coma and died within 2 days. Postmortem—ruptured basilar artery aneurysm. |
| 23 | M | 29 | IV | None | B | Hypertensive. Sudden onset of coma. Admitted to a hospital and died within 1 hour. Postmortem—ruptured basilar artery aneurysm and polycystic kidneys. |
| 24 | M | 15 | IV | RC LC | Mce | Four weeks prior to admission sudden onset of severe head pain with vomiting, weakness of left upper extremity. On day prior to admission recurrent head pain and transient unconsciousness. Admitted in decerebrate state. Progressive downhill course and death on 11th hospital day. Postmortem—ruptured right MCe. |
| 25 | F | 53 | III | RB LC | Lesion R-IC | 2 days prior to admission sudden onset of headache and vertigo, followed by transient loss of consciousness. Confused and lethargic with nuchal rigidity on arrival at another hospital and bloody CSF. Same status on admission. Past history frequent headaches and hypertension. Three days after admission decerebrate phenomena 6 days after admission and death 7 days after admission. Multiple LP's were bloody. Postmortem. |
| 26 | F | 59 | IV | LC | MCe | One week prior to admission sudden onset of headache and confusion. On admission semistupor, asphasia, nuchal rigidity. Progressive downhill course with death on the 11th hospital day. |
| 27 | M | 62 | IV | LC | MCe | Hypertensive. On day prior to admission sudden onset of severe headache with transient unconsciousness. On admission bilateral papilledema, nuchal rigidity. Recurrent hemorrhage on 4th hospital day. He then stabilized. Two neurosurgical opinions were against surgery. On the 13th hospital day recurrent hemorrhage and death. Postmortem—ruptured aneurysm MCe. |
| 28 | F | 54 | IV | LC RC | ACo | Hypertensive. Sudden onset of severe headpain followed by coma. On admission coma with right hemiparesis. Progressive downhill course. Recurrent hemorrhage on 9th hospital day and death. |

TABLE VI—*Continued*

| Case # | Sex | Age | Stage | Angiography | Aneurysm | Summary |
|---|---|---|---|---|---|---|
| 29 | M | 53 | IV | None | ACe | Hypertensive. On day of admission sudden onset of headache followed by coma with right hemiplegia. Died within 3 hours of hospitalization. Postmortem—ruptured ACe. |
| 30 | F | 44 | IV | LC | LMCe<br>RMCe | Sudden onset of coma. On admission no focal findings and died within 2 days. Postmortem—unruptured aneurysms right MCe. |
| 31 | F | 65 | IV | RC | MCe | Sudden onset of coma. Admitted without focal findings. Progressive downhill course with death on 7th hospital day. Postmortem—ruptured right MCe. |
| 32 | F | 40 | III | LC | LC | Protocol missing. Apparently bled within one week of hospitalization. Admitted with signs of meningeal irritation and mild neurological signs and symptoms. Died on second hospital day. |
| 33 | F | 67 | IV | LC | ACe | Hypertensive. Nine days prior to admission sudden onset of headpain with confusional state. Progressive mental syndrome. Admitted in coma. Died within 2 days. Postmortem—ruptured ACe. |
| 34 | F | 47 | III | RC | RMCe | Two weeks prior to admission sudden onset of headache with nausea and vomiting followed by transient unconsciousness. On admission, left pupil larger than right, rectal hemorrhages, left facial paresis. During angiograph developed semistupor and left hemiparesis. Angiography revealed spasms of the middle cerebral artery. Rebled on 4th hospital day and died. |
| 35 | M | 65 | IV | RC | ACe | Hypertensive. Recurrent hemorrhage on 4th hospital day and again on 9th hospital day. Remained semicomatose until death 21 days after admission. Postmortem—ruptured aneurysm ACe (right carotid arteriogram did not demonstrate the aneurysm). |
| 36 | F | 32 | IV | LC | MCe | Died within 24 hours of admission. Postmortem—ruptured MCe. |
| 37 | F | 46 | IV | None | PCo | Sudden onset of coma on day of admission. Patient died within 7 hours. Postmortem—ruptured PCo. |
| 38 | F | 56 | IV | None | B | Hypertensive. Admitted with sudden onset of coma. On admission no focal findings. Died within 24 hrs. Postmortem—ruptured basilar artery. |
| 39 | F | 45 | IV | None | MCe | Sudden onset of coma. On admission no focal findings. Died in 6 hrs. Postmortem—ruptured MCe. |
| 40 | F | 59 | IV | LC RC | PCo | Sudden onset of coma on day of admission. No focal findings but she had nuchal rigidity. Progressive downhill course with death on 14th hospital day. Postmortem-ruptured PCo. |
| 41 | M | 44 | IV | None | ACe | Sudden onset of headache followed by unconsciousness, on day of admission. On admission in coma. Died within 24 hrs Postmortem—ruptured ACe, also unruptured MCe. |
| 42 | M | 66 | IV | LC RB | ACo | Three days prior to admission sudden onset of severe headpain, and confusional state. On admission severe organic mental drome bilateral Babinski signs, nuchal rigidity. Rapid downhill course with death on 5th hospital day. |
| 43 | F | 57 | IV | RB LB | IC ACo | Sudden onset of confusion with semistupor on day of admission. On admission comatose. Progressive downhill course with death on 14th hospital day. Postmortem—ruptured ACo. |
| 44 | M | 33 | IV | None | ACe | On day prior to admission sudden onset of headache, followed by coma. On admission no focal findings. Death within 2 days. Postmortem—ruptured LACe. Also unruptured aneurysm RMCe. |
| 45 | F | 50 | IV | None | ACo | Sudden onset of coma on day of admission. No focal findings on admission. Progressive downhill course and death on 8th hospital day. Postmortem—ruptured ACo. |
| 46 | F | 49 | IV | RC | PCo | Sudden onset of confusion and stiff neck while hospitalized for treatment of Erythema nodosum. Progressive downhill course and death in 11 days. Postmortem—ruptured aneurysm PCo. |

### TABLE VI—*Continued*

| Case # | Sex | Age | Stage | Angiography | Aneurysm | Summary |
|---|---|---|---|---|---|---|
| 47 | F | 69 | IV | RB | ACe | Hypertensive. Sudden onset of coma. On admission no focal findings but she had nuchal rigidity. Progressive downhill course. On 7th hospital day a convulsion ensued. Died on 10th hospital day. Postmortem—ruptured ACe. |
| 48 | M | 59 | IV | None | MCe | Four years previously this patient suffered a subarachnoid hemorrhage with right sided neurological abnormalities. There was spontaneous improvement. Sudden onset on day of admission of coma. On admission comatose state with right hemiplegia and nuchal rigidity. Died on 3rd hospital day. Postmortem—ruptured left MCe. |
| 49 | M | 38 | IV | LC | ACo | Sudden onset of coma. On admission no focal findings. Pr-ogressive downhill course with death on 4th hospital day. Postmortem—ruptured ACo. |
| 50 | M | 43 | IV | LC RC | ACo | Sudden onset of coma. On admission bilateral Babinski sign, nuchal rigidity. Progressive downhill course, with recurrent hemorrhage and death on 5th hospital day. |
| 51 | F | 71 | IV | LC | ACo | Seven days prior to admission sudden onset of pain in the neck and transient unconsciousness. On admission severe organic mental syndrome and right hemimotor sensory findings. She improved within the first month of admission and then suffered subarachnoid hemorrhages. She died one month thereafter. |
| 52 | M | 48 | II | None |  | Sudden severe head pain 2 weeks prior to admission. History of severe liver cirrhosis. On admission nuchal rigidity. On 3rd hospital day sudden onset of coma, with dilated left pupil, papilledema, left hemiparesis. Died within 24 hours. Postmortem—ruptured ACo aneurysm. |
| 53 | M | 50 | IV | None | ACo | Sudden onset of coma. On admission no focal neurological findings. Progressive downhill course and death on 7th hospital day. Postmortem—aneurysm ACo. |
| 54 | F | 38 | IV | None | ACo | Six year history of malignant hypertension. Five previous hospitalizations because of "hypertension." Admitted because of headache, shortness of breath. Blood pressure 240/130, bilateral hypertensive retinopathy, acute pulmonary edema was noted. On 14th hospital day after being treated with bed rest, low salt diet, and Ismelin,® she suddenly complained of a severe headache and developed a convulsion and became comatose. She died 4 hrs. thereafter. Postmortem—ruptured ACo. There was severe bilateral kidney disease. |
| 55 | M | 52 | III | RC LC | PCo | Hypertensive. Following spinal anesthesia for prostate surgery suddenly developed a severe headache with confusional state. He became progressively stuporous and developed a left hemiparesis. Eleven days thereafter he died. Postmortem—ruptured PCo. |
| 56 | F | 64 | IV | RC IC | IC | Hypertensive. Sudden onset on day of admission of semicomatose state. On admission stuporous, bilateral retinal hemorrhages, left hemiparesis. Angiography revealed a mass in the region of temporal lobe. Patient died on 3rd hospital day. Postmortem—ruptured IC. |
| 57 | M | 42 | III | None | B | Four years prior to admission transient left-sided weakness. Subsequently developed confusion and had normal R and L carotid angiograms at another institution. On admission alert but disoriented, nystagmus left sixth and seventh cranial nerve palsies dysarthria, right hemimotor sensory syndrome, right Babinski, left carotid bruit. Patient improved but on 3rd day developed fever of 103 and died suddenly. Postmortem. |
| 58 | F | 53 | IV | RB LC | ACo | Ten days prior to admission onset of confused state. On day prior to admission she developed a convulsion. On admission severe organic mental syndrome, bilateral Babinski signs, nuchal rigidity. On 3rd hospital day, she became unresponsive and died. |
| 59 | F | 50 | IV | RC | RMCe | Protocol missing. Apparently critically ill on admission but not in coma. Hypertensive. Died on 3rd hospital day. |

mission revealed only a complete third nerve palsy. One day after admission she suddenly went into coma. Spinal fluid was bloody. Emergency right and left carotid arteriography revealed a right posterior communicating artery aneurysm and a left posterior communicating artery aneurysm. Patient died the next day.

*Case No. 4.* A 43-year-old woman developed a sudden attack of head pain three weeks prior to admission. She was admitted elsewhere and angiography revealed an anterior communicating artery aneurysm. On admission to The Mount Sinai Hospital she was asymptomatic and there were no neurological findings. Fifteen days thereafter there was a sudden onset of headache, convulsion and death.

## Discussion

Of the four cases, Numbers 1 and 2 clinically had evidence of recent subarachnoid hemorrhage.

Both rebled (No. 1 on the seventeenth hospital day; No. 2 on the ninth hospital day). Both had clinically improved and were relatively well stabilized; the active bleeding had apparently stopped, although xanthochromia was noted. Today many neurosurgeons would probably have operated upon them prior to the rebleeding. Some neurosurgeons would have treated them with 6-aminocaproic acid and hope that the acute episode would abate prior to the second hemorrhage.

Of the latter two patients, No. 3 had bilateral posterior communicating artery aneurysms, the right one having ruptured, and No. 4 had an anterior communicating artery aneurysm. On admission to the hospital these two patients would have been considered ideal cases for intracranial surgery by most neurosurgeons.

When discussing management of intracranial aneurysms there is a veritable confusion since a physician who favors one method of management reflects his own specific experiences which naturally differ from a physician who prefers a different mode of therapy. It is necessary to make comparable analyses in matching one treatment with respect to another.

In this paper to state that 59 of 117 patients with ruptured intracranial aneurysms died under conservative management (that is, a mortality rate of 50 percent as compared to the usually quoted surgical mortality figures of 5 to 35 percent) is meaningless if, as noted above, 55 of these 59 patients would be considered poor (if at all) surgical risks.

This paper does reflect the usual mortality rate of the first incidence of a ruptured intracranial aneurysm. This illness is a most serious one.

This series of 117 patients includes 55 patients whose disease was so severe that death was inevitable, and no currently known method of therapy could save any. Of the remaining 62 cases, 58 survived and 4 died. The mortality rate, after abstracting these 55 doomed cases, was 6.5 percent.

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# Treatment of Ruptured Intracranial Aneurysms by Induced Hypotension

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## Introduction

The use of induced hypotension for the medical treatment of ruptured intracranial aneurysms is increasing because of consistently encouraging results (1–8). Recently, further impetus to the utilization of this method was provided by the randomized Treatment Study-Cooperative Aneurysm Project (9), sponsored by the National Institute of Neurologic Diseases and Blindness (later Stroke). The study had been designed in large part by neurosurgeons and conducted by fifteen medical centers in this country and abroad. For the first time, there was a *random* comparison of the effectiveness of: 1) plain (regulated) bedrest with general supportive measures, commonly referred to as "conservative therapy"; 2) hypotensive therapy; 3) carotid ligation; and 4) craniotomy. "The results of these analyses were that regulated bedrest was the poorest treatment, while drug-induced hypotension was the best treatment with common carotid ligation a close second-best." In the last few years, the importance of lowering blood pressure in order to prevent recurrent aneurysmal rupture has also been proved mathematically (10, 11).

Shortly after its introduction in 1956 (1), the effectiveness of the hypotensive approach in the acute stages of hemorrhage became apparent to us and its application early in the illness was urged, with the suggestion that after the acute stage of illness had been stabilized by hypotensive therapy, the patient's status be reevaluated and a decision made as to whether medical-hypotensive therapy ought to be continued indefinitely without operation or operation should be performed on what had become a better-risk patient (4). Some more recent data on long-term hypotensive therapy now indicates that better results may be obtained if the patient is continued on the medical regimen indefinitely (12).

## Case Material

Ninety-one consecutive patients, all with proved hemorrhage and with proved aneurysm(s) were treated medically by hypotensive therapy (Table I). The advantages of medical treatment over surgical become apparent from the outset insofar as a greater variety of cases may be treated by the former. Selection in medical management is held to the same minimum as before in that the only patient excluded is the persistently uncommunicative one who cannot be treated by this method since titration of treatment is not possible in this instance. (Extremely poor surgical results have already led some surgeons to

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TABLE I

*Case Material*

| Age | Location of Aneurysms* | Number of Aneurysms | Number of Patients with Multiple Aneurysms |
|---|---|---|---|
| less than 50—40 patients | IC** | 50 | 20 patients |
| 50 or more—51 patients; includes 21 patients age 60 or more and of these, 10 patients age 65 or more | ACC | 34 | |
| | MC | 18 | |
| | PERI | 7 | |
| | PC | 3 | |
| | B | 7 | |
| | V | 4 | |
| Totals—91 Patients | 123 Aneurysms | | |

| Interval between Hemorrhage and Start of Treatment | Clinical Condition at Beginning of Treatment | | |
|---|---|---|---|
| | Grade*** | Number of patients | |
| 3 weeks or less—70 patients; including 23 patients treated less than 48 hours from onset of hemorrhage | I | 12 | = good risk (41%) |
| | II | 25 | |
| More than 3 weeks—21 patients | III | 32 | = poor risk (59%) |
| | IV | 22 | |

* IC = Internal carotid, including region of origin of posterior communicating artery.
ACC = Anterior communicating artery region.
MC = Middle cerebral.
PERI = Pericallosal.
PC = Posterior cerebral.
B = Basilar.
V = Vertebral.

** The larger number of IC aneurysms is due at least in part to the greater tendency for multiple aneurysms to occur here and also a tendency for the more difficult cases including multiple aneurysms, to be referred for hypotensive therapy.

*** For comparative purposes, the Botterell surgical classification has been used. It is noted that although patients in Grade V are not treated (recent surgical opinion is adopting this view—see text), 3 of the patients in Grade IV were "close to" Grade V.

stop operating on these cases). No patient has ever been refused hypotensive therapy because of any other reason, for example, being over sixty-five years of age, the presence of spasm, complicating medical illnesses, absence of demonstrable cross-circulation, reaction to carotid compression, inaccessibility of aneurysm, presence of multiple aneurysms, and, especially, recency of bleeding. Yet, these restrictions have kept many of the more difficult cases out of surgical statistics. As an example of the weighting of the medical series with these poorer risk patients, one may consider the age factor. Patients sixty years of age or more are generally avoided by surgeons. However, twenty-one patients, i.e., almost one-fourth of my entire series of ninety-one cases, were sixty years of age or more at the time of hemorrhage and treatment. Another limitation often found in surgical series involves the presence of multiple aneurysms, particularly when four or more aneurysms are demonstrated. Expanded use of angiography has resulted in more adequate visualization of these additional aneurysms. In the present hypotensive series, twenty patients have multiple aneurysms—five patients having four or more aneurysms each.

Further comparison with surgical data may be obtained by using one of the surgical systems (13) of classifying the patient's pretreatment condition (Table I), again demonstrating that many more difficult cases are treated by the medical method than by the surgical methods. In the present medical series, when one considers pretreatment clinical condition, more than half (59 percent) of the total of ninety-one patients were poor risk cases, leaving only 41 percent of the cases in the good risk categories, i.e., Categories 1 and 2. Moreover, it must be remembered that not all of these 41 percent "good risk" patients were actually operable—some were in fact rejected by the surgeons for other reasons, e.g., inaccessibility of aneurysm, or reaction to carotid compression (bilaterally). One may compare this with surgical series wherein, by selection, the percentage of good risk cases, instead of being only forty-one percent of the total, may range from 75–100 percent of the (surgical) series. It is quite clear that in any series, the presence of a large number of good risk cases will yield better results; this is the basis of most surgical results.

Still another comparison involves early treatment—the most difficult time—when mortality is highest. In the present medical series, twenty-three patients were treated within forty-eight hours of the onset of symptoms. One may contrast this with many recent surgical series in which there are few if any patients treated even by the end of the first week. In like manner, patients who would have been excluded from surgical series because of any and all of the other restrictions, such as spasm, have been treated immediately by hypotensive therapy.

Figure 1 shows the angiogram of one of the twenty-five long-term follow-up patients, that is, those who have been followed more than ten years from the time of hemorrhage and hypotensive therapy. This patient, the first one ever treated by the hypotensive method, was sixty-two years old at the time of hemorrhage and treatment. She has been maintained on this regimen for seventeen years, is now seventy-nine years old, and remains in exellent condition—without operation.

## Method

The hypotensive method has been described fully in several publications (1, 2, 4, 6, 14, 15) and so will be only outlined here. Briefly, the blood pressure is brought down by drugs, with or without the use of a tilt table, and the degree of pressor responsiveness is reduced by these drugs. Use of the tilt table may be helpful for the physician who has not used this method previously or in the case of the patient in whom an extremely careful hypotensive titration is indicated. The hypotensive medications used for the first several years were primarily rauwolfia, thiazides, and hydralazine; ganglionic blockers such as pentolinium were employed for a while but were replaced by other, more efficient drugs.

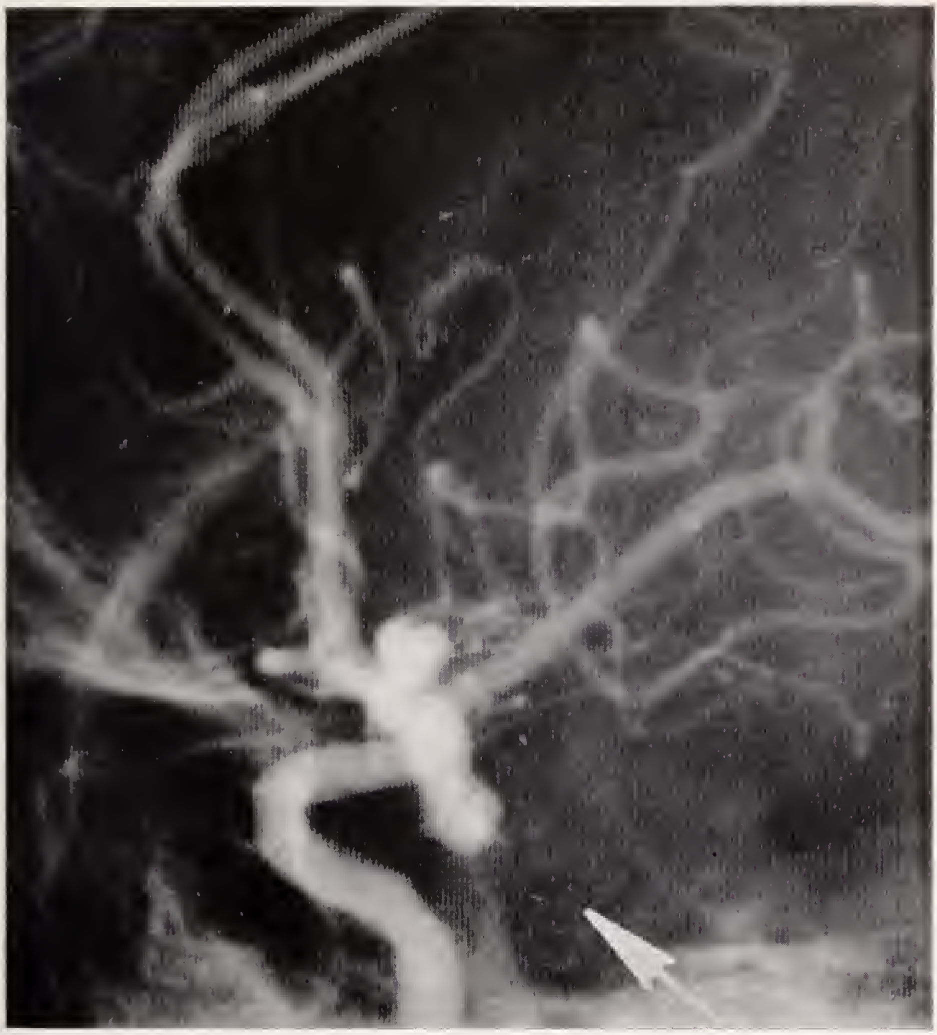

FIG. 1. First patient treated by the hypotensive method. Age 62 at time of hemorrhage and treatment; complete recovery. Treatment maintained for 17 years thus far; continues in excellent health at age 79. Aneurysm of internal carotid—posterior communicating artery.

Recent additions include diazoxide, spironolactone, and alpha-methyldopa; some of these preparations may be less effective than others in long-term follow-up. For example, patients occasionally develop tolerance to alpha-methyldopa. Adjunctive medication such as epsilon-aminocaproic acid to aid clotting is also being evaluated; years ago, vitamin K had been tried in this regard (16, 17). The kind of medication selected is dependent more upon the neurologist's familiarity with the given drug or drug combination; he should become acquainted thoroughly with several. Other medications include anticonvulsants, antacids, stool softeners, and mild analgesics; with this program, stronger analgesics such as morphine and Demerol® have been unnecessary. Support stockings are used at least early in treatment and convalescence. Excellent nursing care is mandatory. Although round-the-clock, private-duty nursing care is probably the ideal therapeutic setting, intensive care units are being used to a greater extent now. Additional help in monitoring the blood pressure may be obtained with electronic devices.

Blood pressure reduction is initiated and continued carefully either until signs of cerebrovascular insufficiency occur or the systolic blood pressure reaches a range of 80–90 mm Hg. If signs of cerebrovascular insufficiency occur, the blood pressure is reelevated slightly until those signs disappear. (The presence of another illness compromising the heart or kidneys will alter the range of blood pressure sought). The blood pressure is then kept at this low range indefinitely. It may be noted that in this method, almost from the start, the systolic pressure has been stressed more than the diastolic because 1) the systolic blood pressure is generally heard more reliably, 2) nurses and physicians have been taught two different end points for the diastolic pressure, namely, sound-disappearance and sound-change, and 3) hypotensive drugs exert a much greater effect on the systolic pressure than on the diastolic. It is the author's impression that diastolic pressure may certainly be important; however, that importance may reside in its role in determining pulse pressure —and reduction of the latter may be especially important in reduction of stress to the aneurysm.

## Results

Seventy-three of the ninety-one patients survived their aneurysm rupture. Table II shows the condition of these seventy-three survivors. It is apparent

TABLE II

*Quality of Survival—73 Patients, Includes 6 who Died Later of Causes not Directly Related to Aneurysm*

| | Excellent* | Good | Fair | Poor |
|---|---|---|---|---|
| No. of Patients | 61 | 8 | 3 | 1 |

* Excellent = No residual defects or only a third cranial nerve palsy.
Good = Mild residua; working or able to work.
Fair = Moderate defects such as mild to moderate organic mental syndrome or a left hemiplegia without an organic mental syndrome.
Poor = (One) patient, age 65, developed repeated (3–4) subarachnoid hemorrhages, a few days apart; multiple bilateral aneurysms were found. She became almost moribund but was treated and recovered with only a slight residual organic mental syndrome; she was able to ambulate without assistance. However, after a few years, gradual worsening began and now, at age 72, there is a marked organic mental syndrome and she requires assistance in standing or walking.

that the quality of survival is generally excellent, with sixty-one out of the seventy-three, or eighty-four percent in this category. If the categories "excellent" and "good" are combined, the result is 95 percent of the total; four percent are "fair" and one percent (one patient) is "poor."

Aneurysm-related deaths are detailed in Table III. Late deaths in follow-up from causes not directly related to aneurysm occured in six patients and were caused by abdominal neoplasm, lobar pneumonia, influenza and pulmonary embolism, coronary occlusion, bronchopneumonia, and influenza with possible myocardial infarction. It is interesting that spasm has not been a significant problem in the hypotensive management of these cases despite its angiographic demonstration in many of the patients. In fact, the patient in category (a) of Table III was the only patient in the entire series whose death was caused by it, and, in this instance, the blood pressure had not been adequately lowered. One might speculate that if these observations are correct, then perhaps there is a protective effect against spasm exerted by some part of the hypotensive regimen, whether related to the hypotension per se or possibly to another drug effect.

TABLE III

*Aneurysm-Related Deaths, 17-Year Total: 18 Deaths out of 91 Patients on Hypotensive Therapy*

| | Up to and including 9 weeks from time of last hemorrhage | | After 9 weeks from time of last hemorrhage | | | |
|---|---|---|---|---|---|---|
| | within first 3 weeks (a) | 3–9 weeks (b) | 3½ mos. (c) | 4½ mos. (d) | 23 mos. (e) | 5 years (f) |
| No. of patients | 8 | 6 | 1 | 1 | 1 | 1 |

(a) 1 due to spasm; remainder to recurrent hemorrhage.

(b) 1 due to pulmonary embolism on second day of ambulation; remainder due to recurrent hemorrhage.

(c) Bizarre electrolyte disturbance including hypernatremia (170); aneurysm embedded in orbital surface of brain; died at age 60.

(d) Gradual deterioration after second hemorrhage.

(e) Only fatal recurrent hemorrhage after 9 weeks follow-up, in entire series of 91 patients; died at age 71; multiple bilateral aneurysms; treatment regimen modified since then (1962).

(f) Did well for 3½ years; then aneurysm (large, adjacent to pons) began acting as mass with progressive brainstem dysfunction and death 5 years after hemorrhage.

Summary of all deaths with regard to operability, per neurosurgical consultation, at time of hemorrhage.

Deaths during first 3 weeks—8 deaths—at least 4 = inoperable.

Deaths during 3–9 weeks—6 deaths—at least 4 = inoperable.

Deaths after 9 weeks—Only 1 fatal recurrent hemorrhage—inoperable; the 3 nonhemorrhagic deaths were all considered inoperable.

## Discussion

Increasing acceptance of hypotensive therapy as the treatment of choice early in ruptured intracranial aneurysm has resulted in a shift in the controversy on management to the later stages of this illness. However, when long-term follow-up is then considered, hypotensive results have again been surprisingly good. Ordinarily, if a patient with ruptured intracranial aneurysm survives in excellent condition, without operation, for ten years or more, many physicians tend to believe that the patient has been unusually fortunate and that the situation is relatively uncommon. This impression has now been proved incorrect. In the present medical series of ninety-one cases, there are to date twenty-five patients with proved ruptured intracranial aneurysms who have been followed ten years or more on hypotensive therapy, without operation, and in twenty-four of the twenty-five, the patient's condition has remained excellent. Moreover, even in the case of the twenty-fifth patient, the aneurysm problem had been well controlled; her condition was the result primarily of continued severe psychiatric disturbance which had already been present for thirteen years prior to her hemorrhage. She died recently at age eighty-one, of lobar pneumonia.

After seventeen years, in the entire series of ninety-one cases, there have been eighteen aneurysm-related deaths for a mortality of 19.8 percent (Table III). There has been no treatment morbidity. An interesting prognostic feature has emerged from this series—if a patient on this regimen is kept from having a recurrent hemorrhage for a period of nine weeks from the previous hemorrhage, the chances for a fatal recurrence become zero.

Thus, the management problem in this illness is narrowing down to a more intermediate or "second stage" since the first, or acute, stage and the third, or long-term, stage appear to be treated better medically. If surgery is to be used in the treatment of ruptured intracranial aneurysm, this second stage may perhaps be the one in which proponents of surgical therapy must try to compete effectively with those of hypotensive therapy. However, this is contingent upon surgical therapy being improved to a point wherein its use, with the well-known attendant hazards of treatment mortality and treatment morbidity, can be vindicated—in comparison with the results of hypotensive treatment. (Attempts to improve surgical methods involve the use of such devices as hyperbaric chambers and dissecting microscopes; medical methods, on the other hand, improve through the use of such innovations as automatic blood pressure monitors, as well as more effective drug combinations.) It may be added that surgical reports based primarily upon "good risk" cases, for example, almost all patients with single aneurysms and less than sixty years of age, are of little or no value because these patients are generally treated adequately medically with hypotension. Surgery may therefore still be considered but only as part of a combined "medical-surgical-medical" approach; this combined therapy was suggested and implemented some years ago (4, 8) and merits reexamination.

## Summary

The treatment of ruptured intracranial aneurysms has been evolving with a resultant increase in adoption of the hypotensive method especially in the early stages of illness. This trend toward medical treatment has been stimulated further by the recent (1972) report of the International Randomized Study in which, after years of debate, surgical methods were finally randomized against hypotensive therapy; the surgical methods failed to live up to surgical expectations. Most physicians have not yet been apprised of these results and it is suggested that these results ought to be made available to them in view of their importance to the general physician and internist, as well as to the neurologist and neurosurgeon. It is clear that future reports of any surgical innovations must now be studied critically, at least until each surgical modification has had its chance to be randomized against hypotensive therapy.

When one analyzes the kinds of cases treated by hypotensive therapy in comparison with those acceptable for surgery, one notes immediately the striking differences, which means that the more difficult cases are treated medically. This is obvious regardless of what is compared—whether it be age, recency of bleeding, pretreatment condition of the patient, presence of spasm, or presence of multiple bilateral aneurysms. The fact that the worst of these situations are consistently found in the medical series is most illuminating when it comes to interpreting results. These discrepancies, it is noted, have held true in every comparison so far and help to explain why surgical results, although generally optimistic, have not been able to equal the results of hypotensive therapy. Thus, when these discrepancies were cancelled out by the randomized study, the results of surgery were extremely disappointing when compared with those of hypotensive therapy.

Besides being effective in the acute stages of illness, hypotensive therapy has now been found to be extremely effective throughout the later stages of illness. Whether the use of surgical therapy will therefore still be justifiable, perhaps for an intermediate second stage of illness, or whether the treatment of ruptured intracranial aneurysms will become entirely medical for all stages, is currently being investigated.

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# Microsurgical Treatment of Cerebral Aneurysms

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The use of the binocular surgical microscope for the operative treatment of intracranial aneurysms has been described by Adams and Witt (1), Pool (2), Rand (3, 4), Yasargil (5), and others (6, 7). The microscope allows stereoscopic visualization with greatly increased illumination and magnification of the operative field. This has resulted in more accurate dissection of the aneurysm neck with improvement of clip placement. It has decreased the probability of aneurysm rupture during dissection. It has diminished the likelihood of injury to adjacent structures surrounding the aneurysm and inadvertent occlusion of the parent vessel or nearby perforators from faulty clip placement. Improved magnification sometimes permits delineation of an aneurysm neck where gross visualization might not suffice. Since dissection is more precise and gentle, there is less postoperative spasm.

Since 1970, we have used the operating microscope for all cerebral aneurysm cases. The present series consists of 42 consecutive patients operated on by the authors between January 1970, and October 1972. The ideal criteria for operability were normal cerebrospinal fluid pressure and good level of consciousness. However, all grades of patients were accepted for operation unless they were moribund (Grade V). No patients were excluded because of age, location of aneurysm, or multiplicity of aneurysms. Not included are several patients operated on for other reasons (pituitary apoplexy and meningioma), who had incidental clipping of an aneurysm during surgery. Patients who died from rebleeding of the aneurysm while being evaluated for or awaiting craniotomy are not included.

The time of operation in relation to the last subarachnoid hemorrhage varies considerably in this series. An attempt was made to operate between ten and 14 days after initial aneurysm rupture. Delay of operation was occasionally advised when the patient's condition deteriorated because of cerebrovascular spasm. A few patients were not transferred to us until a number of weeks after their subarachnoid hemorrhage. If deterioration of neurological status was thought to be caused by a life-threatening intracerebral hematoma, emergency craniotomy was performed.

Follow-up period varies considerably since these are consecutive cases. Some patients have been observed for approximately two years, but more recent cases only for months. Postoperative results are graded according to the most recent evaluation of the patient.

## Grading of Aneurysms

The preoperative classification of patients follows that recommended by Botterell et al (8).

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*Favorable Risk*

Grade I. Patients who are conscious with or without signs of blood in the subarachnoid space.

Grade II. Drowsy patients without significant neurological deficit.

*Poor Risk*

Grade III. Drowsy patients with significant neurological deficit and probably an intracerebral clot.

Grade IV. Patients with major neurological deficit and deteriorating because of large intracerebral clots, or older patients with less severe neurological deficit but preexisting degenerative cerebrovascular disease.

Grade V. Moribund or nearly moribund patients with failing vital centers and extensor rigidity.

Results of surgery are graded as follows:

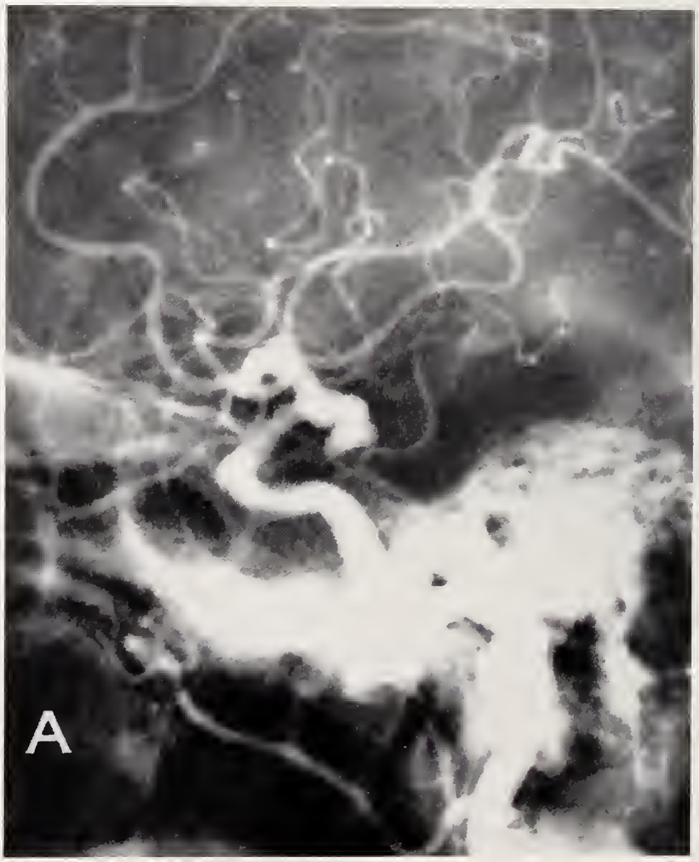

Fig. 1a.

Fig. 1. Internal carotid artery aneurysm. a. Preoperative lateral view. b. Postoperative lateral view.

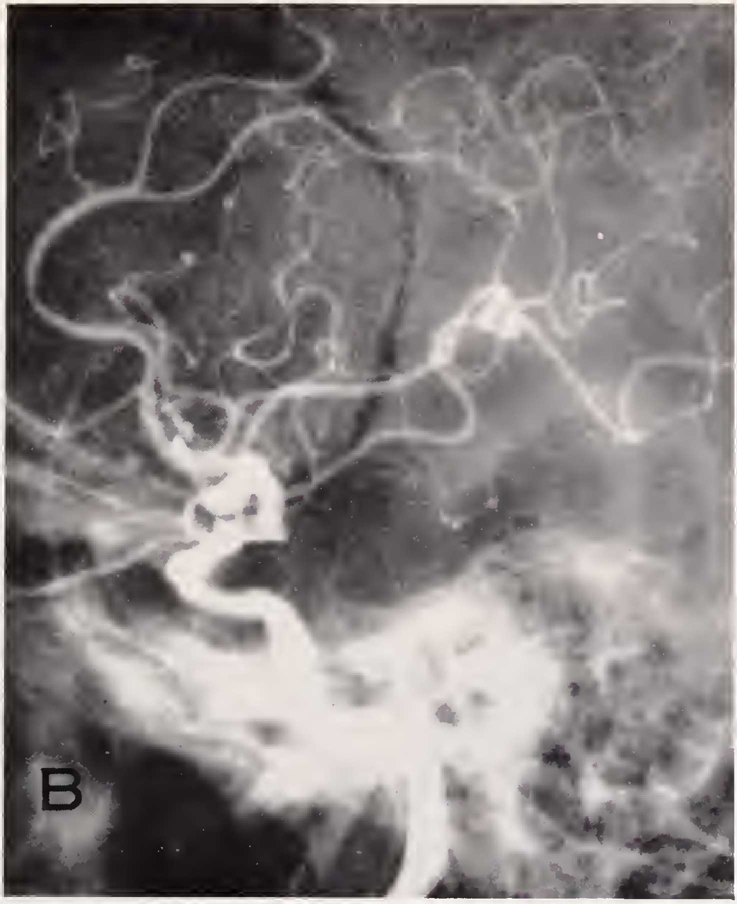

Fig. 1b.

*Normal.* No neurological signs or symptoms other than residual preoperative third nerve paresis.

*Good.* Minimal neurological signs and symptoms with no significant hemiparesis or mental changes. Able to participate in usual activities and work.

*Fair.* Moderate hemiparesis or slight mental changes but able to work or partially participate in most usual activities.

*Poor.* Hemiplegia or severe organic mental changes. Unable to work or participate in most usual activities.

*Dead.* Includes all patients with surgically related deaths at time of operation or thereafter. Also includes patients who died from rebleeding of aneurysm after hospital discharge.

### Internal Carotid-Posterior Communicating Artery Aneurysms

There were 20 patients with aneurysms of the internal carotid artery, mostly at the junction of the posterior communicating artery (Figs. 1a and 1b). Preoperative status and postoperative results are summarized in Table I. Technically, this was the simplest operation and involved the least amount of dissection and exposure. All aneurysms in this location were occluded with a single Heifetz clip (9). No aneurysm ruptured during surgery.

Postoperative angiography was performed in 19 cases and demonstrated complete occlusion of the aneurysm with patency of the internal carotid artery in 18 patients. In one patient, a small portion of the neck was visualized.

The major problem was postoperative spasm which resulted in hemiparesis in four patients. However, there was significant improvement of hemiparesis in every case where this complication occurred. Three of the four patients with

TABLE I

*Aneurysms of Internal Carotid-Posterior Communicating Artery*

| Patient | Age | Interval between Last Bleeding and Surgery | Preoperative Grade | Postoperative Result |
|---|---|---|---|---|
| B.T. | 51 | 9 days | 1 | F |
| L.P. | 29 | 12 days | 1 | N |
| H.A. | 49 | 14 days | 1 | G |
| M.O. | 67 | unruptured | 1 | N |
| M.V. | 23 | 14 days | 2 | N |
| R.M. | 44 | 20 days | 1 | N |
| B.G. | 37 | 14 days | 3 | N |
| H.H. | 51 | 29 days | 3 | G |
| M.B. | 67 | unruptured | 1 | N |
| R.G. | 32 | 8 days | 3 | N |
| J.G. | 56 | 23 days | 2 | N |
| M.M. | 35 | 18 days | 1 | N |
| E.G. | 38 | 10 days | 2 | F |
| T.S. | 42 | 7 days | 2 | G |
| L.B. | 49 | unruptured | 1 | N |
| L.M. | 31 | 12 days | 1 | N |
| A.H. | 57 | 13 days | 1 | N |
| T.G. | 45 | 10 days | 1 | N |
| G.C. | 37 | unruptured | 1 | N |
| V.C. | 48 | 16 days | 1 | N |

(N = Normal; G = Good; F = Fair; P = Poor; D = Dead).

TABLE II

*Aneurysms of Middle Cerebral Artery*

| Patient | Age | Interval between Last Bleeding and Surgery | Preoperative Grade | Postoperative Result |
|---|---|---|---|---|
| E.G. | 40 | 14 days | 4 | G |
| S.F. | 47 | 13 days | 1 | N |
| M.F. | 55 | 40 days | 1 | N |
| A.D. | 44 | 12 days | 1 | N |
| P.J. | 23 | 1 day | 4 | N |

postoperative spasm improved to the point where they were able to work or carry out normal household activities. There was no morbidity in the four cases where the patient had a third nerve palsy but no subarachnoid hemorrhage. There was no postoperative mortality in this group of aneurysm patients.

## Middle Cerebral Artery Aneurysms

Five patients with aneurysms of the middle cerebral artery were operated upon. The results are summarized in Table II. Exposure of the aneurysm was obtained by dissecting distal to the internal carotid artery along the main trunk of the middle cerebral artery to its trifurcation.

Preoperatively, it was often difficult to tell if an aneurysm neck was present. However, at surgery, all aneurysms in this group were found to have a neck across which a clip or ligature could be placed. Without adequate illumination and magnification, some of these aneurysms would probably not have been considered to have adequate necks and would have required wrapping.

There was no morbidity or mortality in this group. In every case, postoperative angiography showed complete occlusion of the aneurysm with preservation of the parent vessel.

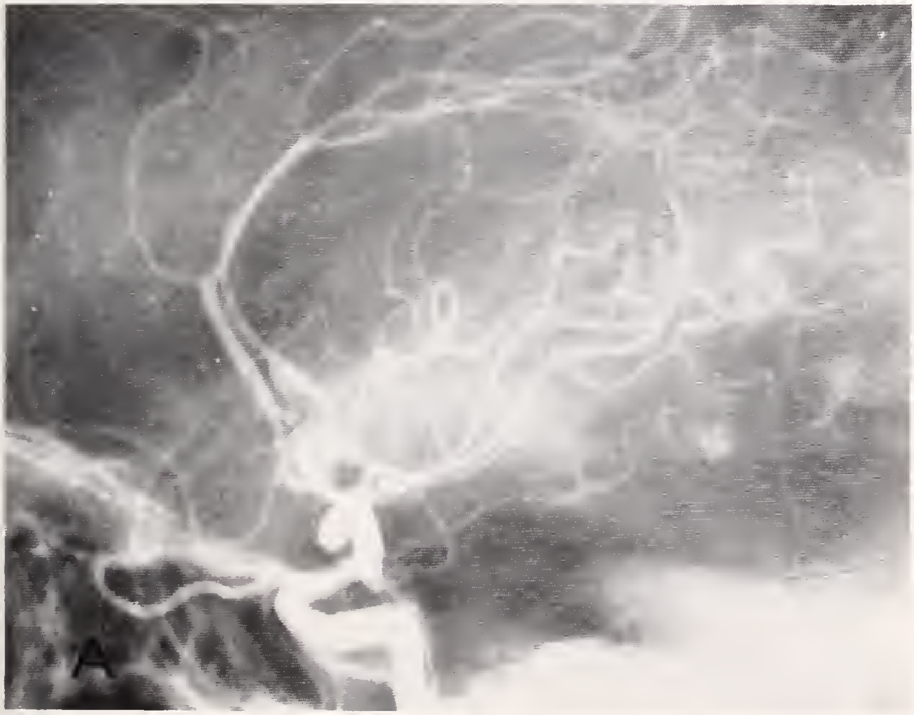

Fig. 2a.

Fig. 2. Anterior communicating artery aneurysm. a. Preoperative lateral view. b. Preoperative AP view. c. Postoperative lateral view. d. Postoperative AP view.

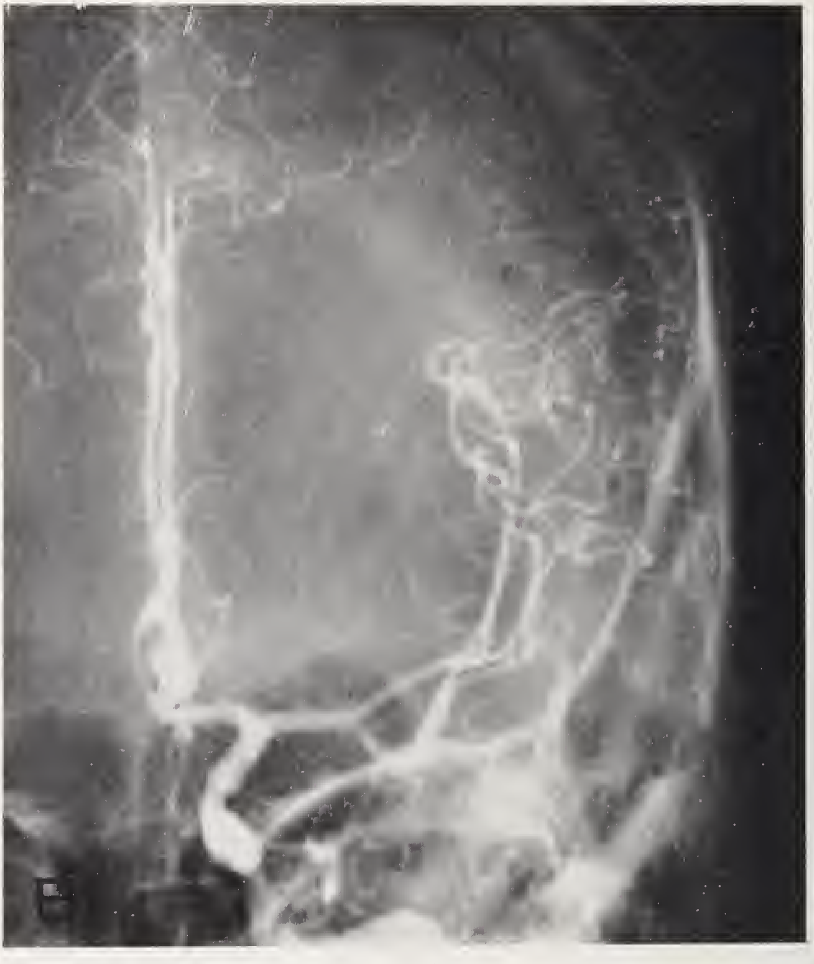

FIG. 2b.

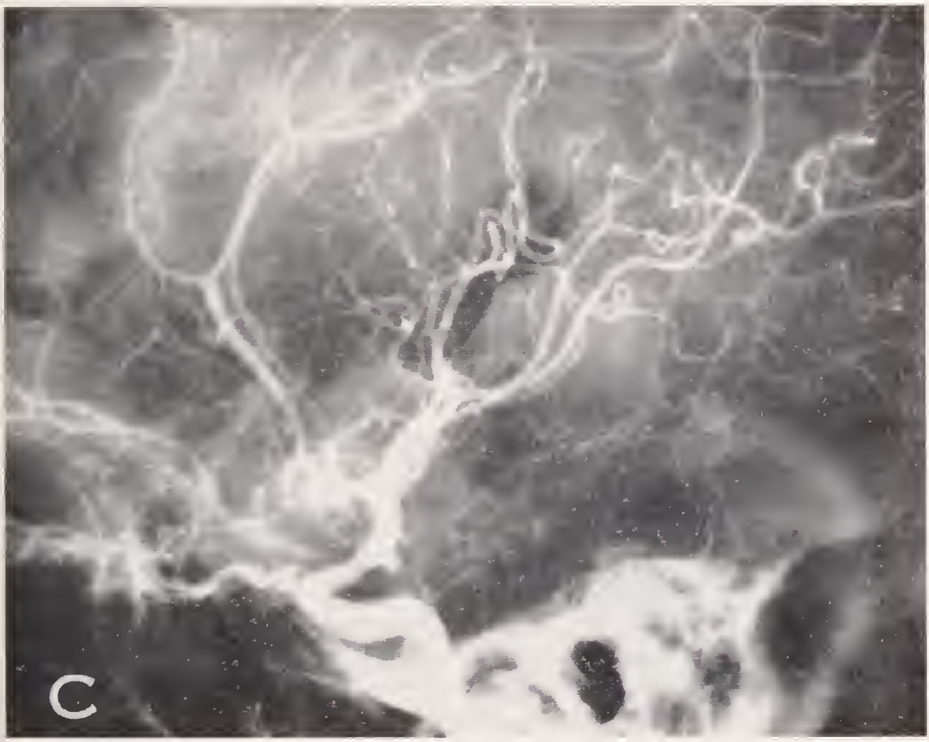

FIG. 2c.

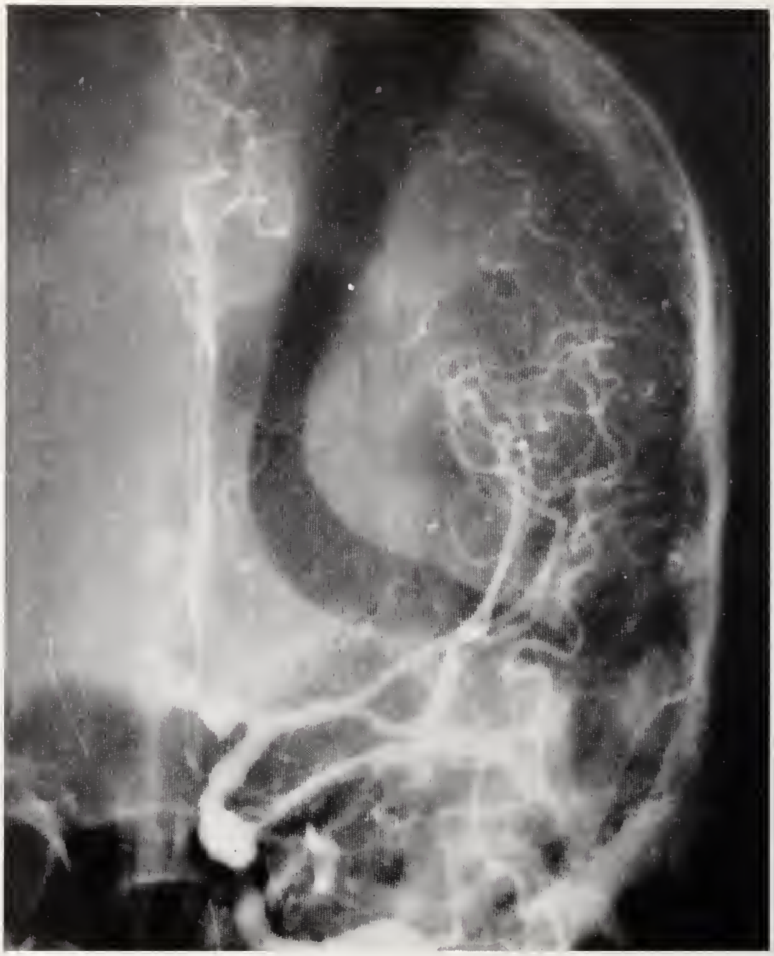

FIG. 2d.

## Anterior Cerebral-Anterior Communicating Artery Aneurysms

Twelve patients had aneurysms of the anterior cerebral or anterior communicating artery (Figs. 2a–2d). The results are summarized in Table III. These aneurysms presented the most technical difficulties, especially if large and pointing posteriorly. Smaller aneurysms, which pointed down and forward, were relatively easy to clip. A unilateral frontal bone flap was satisfactory for forward-pointing aneurysms. A bifrontal approach was usually used for aneurysms which pointed backward. In this group, as in aneurysms in other locations, temporary occlusive clips were not found to be necessary. Eleven cases were clipped and one wrapped with muslin and aneuroplastic material.

One patient who was preoperatively Grade IV, had a poor result. There were two deaths. One patient with a large backward-pointing anterior communicating artery aneurysm died shortly after surgery. This was the only instance of aneurysm rupture during surgery. A second mortality resulted from severe postoperative spasm.

Postoperative arteriography was performed in ten patients. In all but one case where clipping was performed, there was complete obliteration of the aneurysm. In the single patient who had some filling of the aneurysm neck on

TABLE III

*Aneurysms of Anterior Cerebral-Anterior Communicating Artery*

| Patient | Age | Interval between Last Bleeding and Surgery | Preoperative Grade | Postoperative Result |
|---|---|---|---|---|
| H.H. | 13 | 11 days | 1 | N |
| L.D. | 48 | 32 days | 4 | F |
| W.D. | 50 | 15 days | 4 | N |
| E.R. | 62 | 14 days | 1 | N |
| F.B. | 59 | 10 days | 4 | P |
| J.O. | 69 | 14 days | 4 | G |
| M.W. | 44 | 9 days | 2 | G |
| L.F. | 52 | 21 days | 1 | N |
| B.H. | 45 | 9 days | 1 | D |
| E.W. | 52 | 6 days | 1 | N |
| M.F. | 39 | 11 days | 1 | D |
| A.F. | 37 | 30 days | 1 | N |

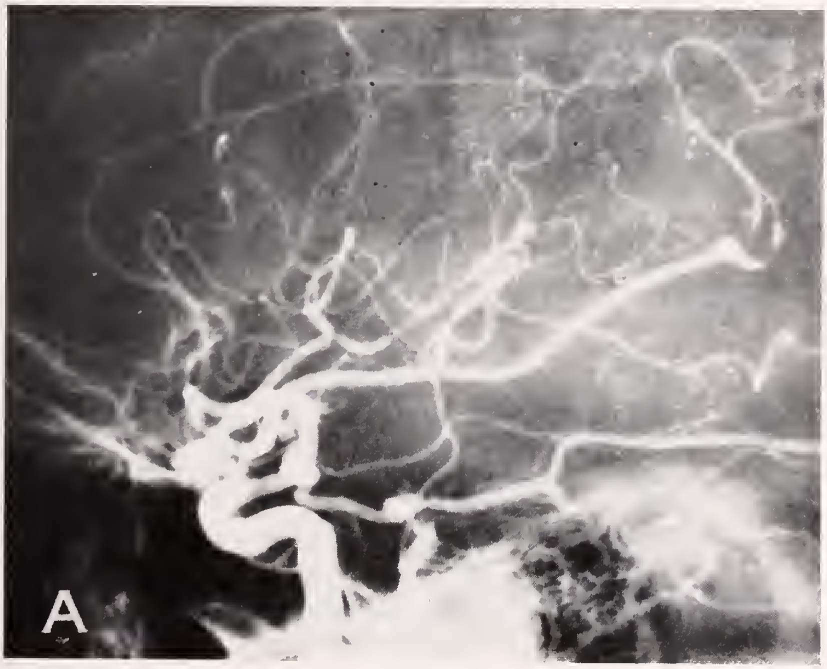

Fig. 3a.

Fig. 3. Multiple aneurysm (middle cerebral and internal carotid arteries). a. Preoperative lateral view. b. Postoperative lateral view.

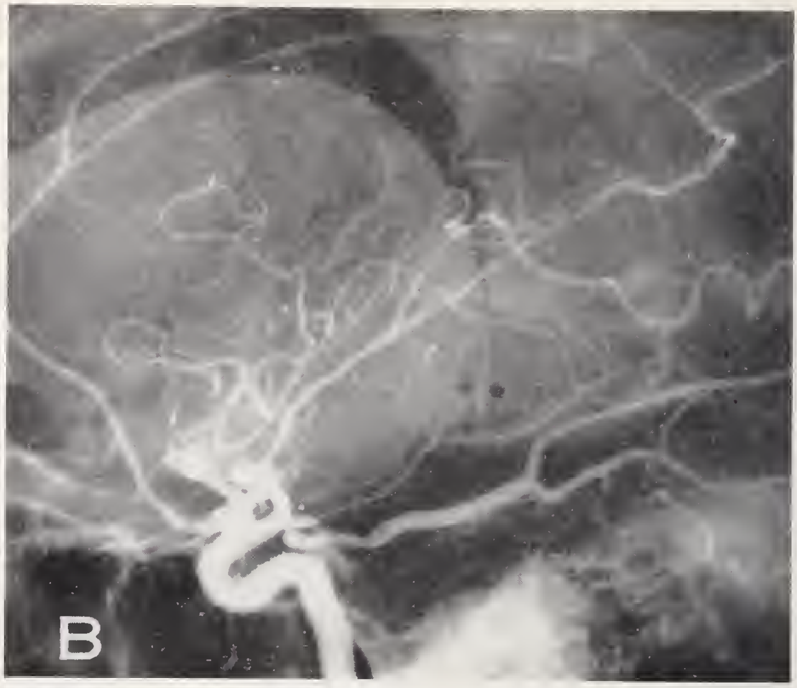

Fig. 3b.

TABLE IV

*Multiple Aneurysms*

| Patient | Age | Location of Aneurysm | | | Interval between Last Bleeding and Surgery | Preoperative Grade | Postoperative Result |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | |
| L.P. | 45 | ICA | MCA | MCA | 26 days | 1 | N |
| R.T. | 49 | ICA | MCA | — | 30 days | 4 | F |
| S.S. | 60 | ICA | MCA | — | 12 days | 1 | N |
| J.I. | 38 | ICA | prox. ACA | — | 13 days | 1 | N |
| J.M. | 59 | MCA | MCA | — | 17 days | 1 | N |

(ICA = Internal carotid-posterior communicating artery; MCA = Middle cerebral artery; ACA = Anterior cerebral artery).

the postoperative arteriogram, follow-up study two months later demonstrated complete occlusion. Normal cerebral circulation was present angiographically in eight patients. In one case, the proximal anterior cerebral artery was intentionally clipped because the aneurysm had a broad base. This patient had no postoperative neurologic deficit because of adequate cross-flow from the opposite anterior cerebral artery. Another patient had postoperative occlusion of the anterior cerebral artery distal to the clip.

## Multiple Aneurysms

There were five patients with multiple aneurysms (Figs. 3a and 3b). Results are summarized in Table IV. These aneurysms were unilateral and could be clipped at a single operation. We have not operated on patients with multiple aneurysms on opposite sides or combination of supra- and infratentorial aneurysms. These would require more than one operation. Since dissection of the internal carotid artery precedes exposure of the middle cerebral or anterior cerebral artery, it is technically not difficult to clip multiple aneurysms in these locations. One patient had three aneurysms which were successfully obliterated (Figs. 4a–4c).

There was no morbidity or mortality in this group. Four of the five patients had postoperative angiograms. All demonstrated successful clipping of the aneurysms and preservation of normal cerebral circulation.

## Summary and Conclusions

The binocular surgical microscope was used in consecutive operations on forty-two patients with cerebral aneurysms. Postoperatively neurological signs

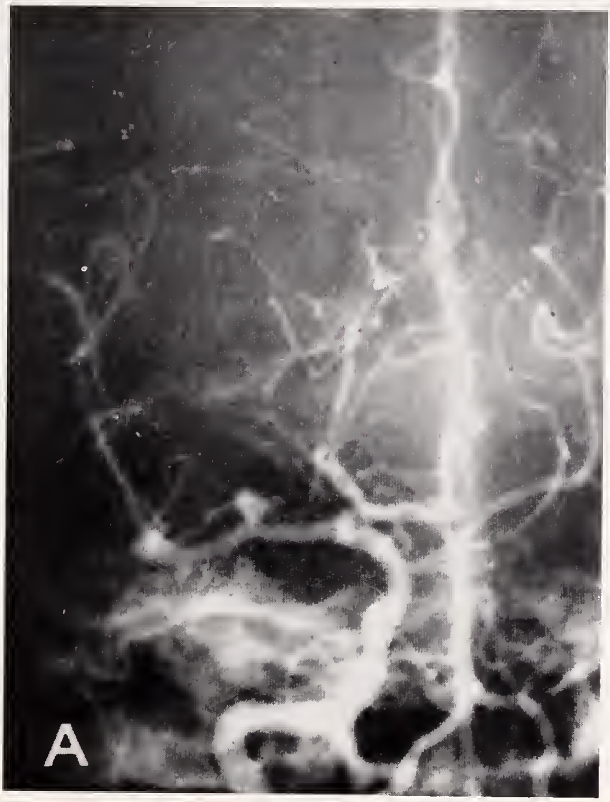

FIG. 4a.

FIG. 4. Multiple aneurysms. Two middle cerebral aneurysms (main trunk and trifurcation) and small internal carotid artery aneurysm. a. Preoperative AP view. b. Postoperative AP view. There is some shift of the anterior cerebral artery, but patient was asymptomatic. c. Postoperative lateral view.

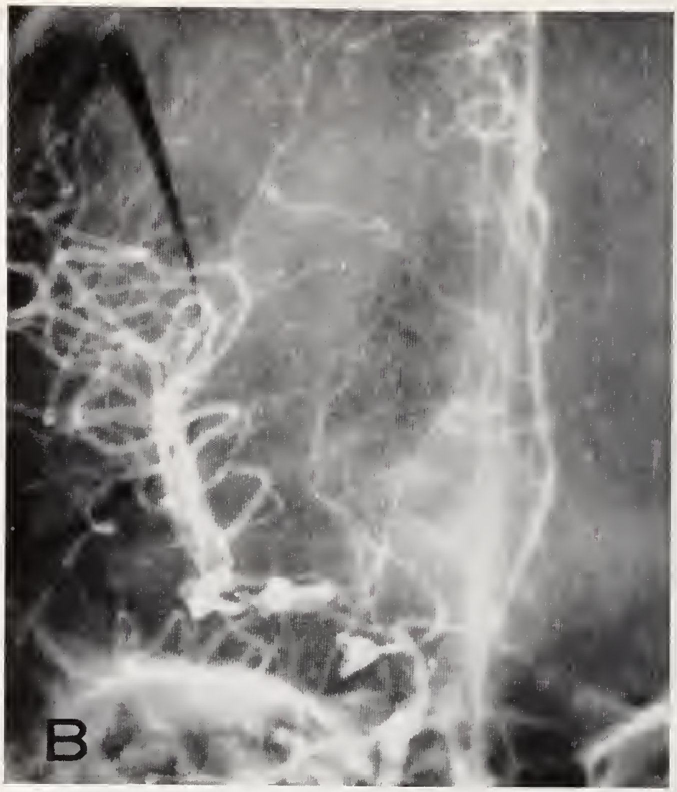

FIG. 4b.

or symptoms were absent or minimal in 83 percent. Fair results were obtained in ten percent. One patient (two percent) had a poor result. Three of the five patients with poor or fair results had significant preoperative morbidity (Grade IV). Two patients died (five percent mortality).

Only one aneurysm ruptured during surgery. No transfusions were required in forty-one cases. There were no infections.

Postoperative angiograms demonstrated a high degree of accuracy in obliteration of the aneurysm. Usually, a single well-placed clip was necessary. It is of interest that in all but two cases, a clippable neck was found. Although some aneurysms are broad-based, the microscope may enable delineation of an aneurysm neck which might not be seen under gross vision.

None of the clips slipped postoperatively. No patient had a recurrence of hemorrhage from rebleeding of aneurysm following surgery.

In our opinion, the use of the dissecting microscope has significantly improved the results of craniotomy for cerebral aneurysms and should be standard procedure for this operation.

### Acknowledgment

The authors wish to thank Dr. Leonard I. Malis for his encouragement and instruction in the use of the microscope for aneurysm surgery.

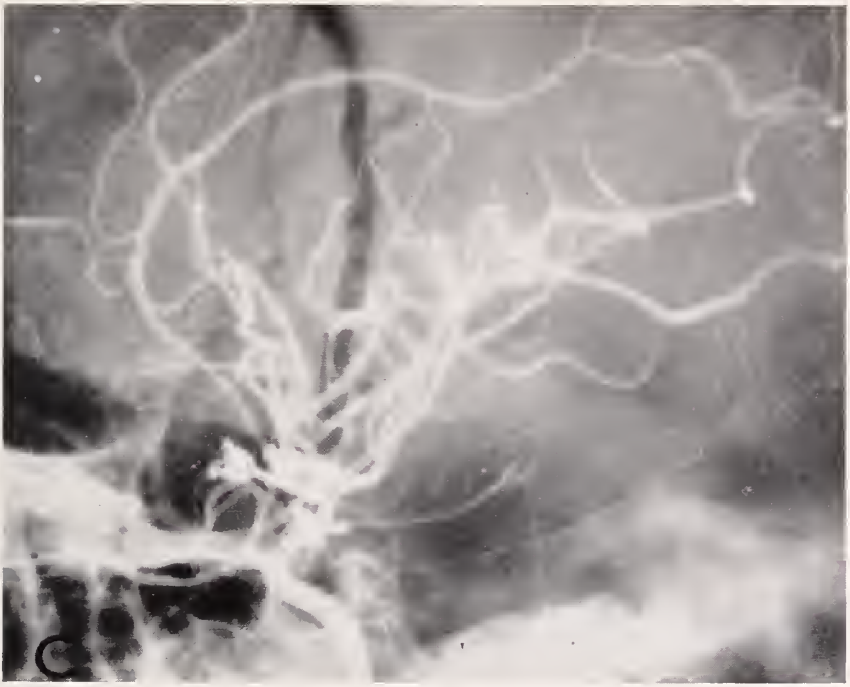

FIG. 4c.

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# Panel Discussion

## Three Therapeutic Modalities for Ruptured Cerebral Aneurysms—Conservative, Hypotensive, and Surgical

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*Moderator*

Lester R. Tuchman, M.D.
Editor, The Mount Sinai Journal of Medicine

*Dr. Tuchman:* The question of the treatment of ruptured cerebral aneurysms is still moot. The Journal has the privilege of presenting from the same institution three well-studied series of good size, treated in very different ways. The participants will now discuss their three papers. The ground rules are as follows:

You have read each others' papers with care. You may challenge as incorrect any fact which you are able to prove is incorrect. You may offer alternative interpretations, but must allow opposing points of view, even if unwelcome to you. You are urged to speak freely and frankly, limited only by the dictates of good taste.

*Dr. Slosberg:* Dr. Sencer, in your paper you mention 117 patients and a 6.5 percent mortality, i.e., four out of 59. It would seem that these 59 should be identified as the 59 who were considered the "good risk" cases of the 117. Is this correct?

*Dr. Sencer:* This is correct. Of 117 patients, 59 died during initial admission, and 58 survived. Four of the 59 who died might have been considered surgical cases; the remaining 55 were not. Therefore, the mortality figures should be four of 62 (58 + 4) from initial admission, or 6.5 percent.

*Dr. Hollin:* I am pleased that a discussion of the preceding papers has been arranged. There ought to be more frequent candid exchanges between neurologists and neurosurgeons, especially when their views might appear to be so diametrically opposed.

I would like to comment on Dr. Slosberg's quotation concerning the results of random comparison of the effectiveness of various modes of treatment. He notes that the "results of these analyses were that regulated bedrest was the poorest treatment, while drug-induced hypotension was the best treatment, with common carotid ligation 'a close second-best.' " This does not apply to the present microsurgical treatment of aneurysms. The results of craniotomy from various centers differ widely from five percent to over 50 percent mortality rate. During the years which this study was in progress, many advances in neurosurgical techniques occurred. These include cerebral dehydrating agents, steroids, improvements in anesthesia, and better instrumentation. Probably the most significant technical advance has been the use of the operative dissecting microscope. With this technique, it is possible to have an overall mortality of about five percent. In my opinion, the statement quoted is incorrect if there is random comparison with the results of craniotomy performed by neurosurgeons competent in the use of the microscope.

*Dr. Slosberg:* The randomized study was very recently published and represents the best results available at fifteen medical centers throughout the country and abroad. The techniques used were both medical and surgical. Surgical innovations are always welcome. Just as it is expected that craniotomy methods will improve, in like manner it is expected that the results of hypotensive therapy will improve also. For example, in my own experience with hypotensive therapy, the mortality is approximately one-half that obtained in the randomized study. Furthermore, the results in long-term follow-up in the past 17 years have been, I believe, superior to the results of surgical long-term follow-up.

With regard to the most recent surgical innovation, that is, microsurgery, randomization of this technique with the technique of hypotensive therapy would probably be worthwhile.

*Dr. Decker:* Dr. Slosberg mentioned that the medical centers involved in the recent cooperative study were certainly major medical centers. It is a fact that none of these centers used microsurgery techniques routinely by neurosurgeons specially trained for aneurysm surgery.

We acknowledge that Dr. Slosberg's published results in hypotensive therapy are better than the cooperative studies. This appears to be partly based on one individual's dedication to this type of therapy. However, one must also consider the fact that he reports approximately 20 percent mortality in a nonconsecutive series because of the exclusion of very sick patients who were not candidates for his therapy. This compares unfavorably with our five percent mortality utilizing the microscope technique.

*Dr. Slosberg:* In terms of the definition of cases treatable by hypotensive therapy, it should be noted that hypotensive therapy is applicable to and has been applied to many more kinds of cases than can be treated by surgical methods. The only cases excluded from hypotensive therapy since its beginning in 1956 have been the patients who are generally classified by Botterell as Category V; the latter group is now considered to be inoperable by many surgeons, which, incidentally, means that all surgical series reported are non-consecutive ones, i.e., at least some, if not all, Category V patients are excluded.

Cases treated by hypotensive therapy consistently comprise much more difficult groups than do those treated by surgery. This was once again demonstrated by the randomized study wherein the differences in groups were eliminated by randomizing. The finding was that hypotensive therapy results were better than surgical results. I expect that, with time, Dr. Hollin's results will follow the same pattern.

*Dr. Hollin:* In our series, ten of the 42 patients were Categories III and IV.

*Dr. Slosberg:* That leaves 32 that were not, i.e., about 75 percent of your cases were good risk in contrast with my cases in which only 41 percent were good risks.

*Dr. Hollin:* That may be so but we had no mortality in any of our ten "poor risk" cases.

Dr. Slosberg stated that medical treatment has an advantage over surgical treatment because of the greater variety of cases that may be treated. While it is true that we might not operate on an elderly patient or one who is in heart failure, extremely debilitated, or has other severe medical complications, some of the conditions listed by him as exclusions from surgery are no longer valid. For instance, absence of demonstrable cross-circulation, reaction to carotid compression, and recent bleeding, may not be absolute contraindications per se, although they might influence the timing of surgery.

We prefer to operate from ten to 14 days following the subarachnoid hemorrhage. If there is no major spasm and the spinal fluid pressure is normal and the patient is alert, earlier operation may be performed. Even if there was spasm on the angiogram, if the patient's clinical condition was good in other respects, surgery could be considered.

We have several elderly patients in our series. The oldest patient was 69 and she was a poor risk (Category IV) prior to surgery. She had adverse reactions following preoperative angiography. She has subsequently gone on to make a good recovery.

The so-called "inaccessibility of an aneurysm" is a rather misleading term because virtually all aneurysms are accessible. It is true that some aneurysms may have a broad base and an unclippable neck. But, these aneurysms could be wrapped rather than clipped.

Multiplicity of aneurysms does not necessarily exclude operation, since once the vascular tree is exposed, several aneurysms of the carotid and its branches may be clipped at one sitting if unilateral. We have successfully operated on one patient with three aneurysms. If aneurysms are bilateral, more than one operation is required. If a patient had four or five aneurysms which were bilateral and in a combination of supra- and infratentorial locations, hypotensive treatment would probably be the method of choice.

One other point regarding the selection for hypotensive medical management should be mentioned, namely, that persistently uncommunicative patients are excluded. This automatically eliminates many Grade IV and Grade V patients. Although Grade V (moribund) cases are not accepted for surgical treatment, we would not turn down an uncommunicative patient in Grade IV, if we thought that the patient had potential for improvement. Younger patients who are aphasic after a subarachnoid hemorrhage have the same potential for improvement as those who have a cerebrovascular accident from other causes. One of our patients with an anterior communicating aneurysm was aphasic and hemiplegic preoperatively, but has excellent postoperative improvement of aphasia.

*Dr. Slosberg:* First, it should be noted that Category V is the only category excluded from hypotensive therapy. Hypotensive therapy has been applied successfully to many patients in Grade III, and also in Grade IV, because only minimal communication is required. Second, with regard to age, although surgical treatment has indeed occasionally been applied to a patient over 60, that patient is usually the exception. This is in marked contrast with the hypotensive therapy series in which almost one-quarter of the entire series was comprised of patients over 60. Third, it is emphasized that, with reference to early treatment, the most acute stage of hemorrhage, i.e., 24 to 48 hours after the bleed, is the time, I believe, when treatment should be started. There are very few surgical series which include such cases. With hypotensive therapy, we have been able to treat 23 such patients, i.e., one-fourth of the entire series. Fourth, with regard to inaccessibility, there are cases in which it is still felt that an aneurysm, for example, a large basilar artery aneurysm compressing the pons ventrally, is simply not operable. Fifth, it is not unusual for patients with multiple bilateral aneurysms to have four, five, or more such aneurysms. In the present hypotensive series, for example, there are 20 cases of multiple aneurysms, including five patients having four or more aneurysms each.

*Dr. Decker:* As far as the timing of the surgery is concerned, Dr. Hollin mentioned that a Grade II patient without spasm on angiography and a normal cerebrospinal fluid pressure may well be a good candidate for early surgery. However, it is known that spasm may develop toward the end of the first week following subarachnoid hemorrhage. Therefore, the early absence of spasm by angiography may be somewhat misleading to the surgeon. For this reason, surgery is usually delayed until the end of the second week following hemorrhage to eliminate the above factors.

It seems that hypotensive therapy with or without the use of antifibrinolysins reduces the incidence of recurrent bleeding during the first few weeks after subarachnoid hemorrhage. However, there does not seem to be any reasonable proof that long-term hypotensive therapy will give statistically superior results to no therapy at all. It has been shown that the risk of rebleeding of an aneurysm after three months following hemorrhage is very low.

*Dr. Slosberg:* It has been my experience concerning the tendency to rebleed that, when a patient on hypotensive regimen reaches nine weeks from the day of hemorrhage, his chances for a fatal recurrence are approximately zero. This is better than a prognosis of "very low recurrence," either when no treatment or surgical treatment has been applied.

With regard to long-term hypotensive therapy, it should be reemphasized that there are now 25 patients who have been followed for ten years or more, and, of these 25, 24 remain in excellent condition, the 25th having died recently at the age of 81 of lobar pneumonia.

*Dr. Hollin:* I would like to point out that none of the aneurysms in our series rebled postoperatively. Satisfactory clipping of the aneurysm was demonstrated on the postoperative angiogram in virtually every case. I believe that if an aneurysm is well clipped there will be long-term protection from rebleeding. If recurrent hemorrhage occurs after craniotomy, it is because the aneurysm was not properly clipped at the initial operation, or that another aneurysm bled.

*Dr. Sencer:* I think that there is a difference between a patient with preexisting hypertension and a patient who develops intracranial bleeding and resulting hypertension. Such patients who were not treated by the hypotensive technique described by Dr. Slosberg frequently have within a week or two normal pressures or a significant drop in blood pressure. Dr. Slosberg's recurrence rate surpasses that of conservative therapy.

I disagree with Dr. Hollin's indications and contraindications for surgery. Aneurysms involving the circulation of the dominant hemisphere, and those which arise from a major blood vessel like the internal carotid or middle cerebral (rather than from a smaller branch) have different potential morbidity. For example, aneurysms "of the posterior communicating artery" are more dangerous for "clipping" when they come off the internal carotid artery rather than the posterior communicating artery per se.

Due to the danger of arterial spasm with resultant cerebral infarction, I strongly question even doing angiography, let alone surgery, in a patient with active intracranial bleeding as evidenced by bloody spinal fluid or xanthochromia. With reference to multiple aneurysms, irrespective of location, I question the wisdom of clipping aneurysms which have not been proven clinically or angiographically to have caused the patients' symptomatology. It is well known that unruptured aneurysms present themselves not uncommonly at the postmortem examinations for other causes. In my experience, second hemorrhages from a different aneurysm are not common.

I wonder about hypotensive therapy as practiced by Dr. Slosberg in the context of the pychological sequelae of such inordinate emphasis on blood pressure as it affects the patient's life thereafter. The issue is the critical three or four weeks after subarachnoid hemorrhage. It is now known that after this period of time, irrespective of conservative, "Slosberg" hypotensive, or surgical therapy, the mortality statistics drop a great deal.

*Dr. Slosberg:* The psychiatric complications of subarachnoid hemorrhage in patients after no treatment or after surgical treatment have been well documented. In my opinion, the patient's awareness that on the hypotensive regimen he now has approximately a zero percent chance for a fatal recurrence is far more reassuring than anything else that can be told him.

*Dr. Sencer:* Dr. Slosberg, I don't believe you understand the difference between the manifest content and latent content from the psychological point of view.

*Dr. Slosberg:* I assure you I do.

*Dr. Hollin:* I wish to thank Dr. Sencer for mentioning some points which I thought were apparent. We don't clip the parent vessel. Of our 42 cases, the parent vessel was intentionally clipped in one case, namely, a proximal anterior cerebral artery aneurysm with a broad base, in a patient who had adequate cross-flow and subsequently no postoperative deficit. In every other case but one, postoperative angiography showed patent parent vessel and clipping of aneurysms. It is true, however, that even if the vessel is patent postoperatively, if the lesion is on the dominant hemisphere and postoperative spasm results, aphasia may occur.

I agree with Dr. Sencer regarding the low incidence of bleeding in cases of multiple aneurysms from aneurysms which had never ruptured. This fact tends to negate one of the arguments for conservative treatment for multiple aneurysms. These cases are not necessarily "inoperable" if it can be determined which aneurysm probably ruptured. However, once the aneurysm is exposed, it may be relatively simple under the microscope to clip the additional aneurysms if they are on the same side.

*Dr. Slosberg:* The previous impression that only the ruptured aneurysm in a case of multiple aneurysms is significant is not completely accurate. Some of the Scandinavian neurosurgeons have indicated a nine or ten percent recurrent hemorrhage rate, the hemorrhage having occurred in previously unruptured aneurysms.*

*Dr. Sencer:* It is significant that Mr. Wiley McKissock, the neurosurgeon-in-chief of the Atkinson-Morley hospital to which most aneurysm patients in Southern England are transferred, has concluded that the intracranial approach to aneurysm is not advisable.

*Dr. Hollin:* Others disagree with Mr. Mckissock.

Dr. Sencer, in the protocol of the 59 itemized patients, can we assume that if it was not specifically mentioned, the patients did not have an intracerebral hematoma or communicating hydrocephalus?

*Dr. Sencer:* If hematoma were present, it would have been noted in the protocol.

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* Heiskanen, O., and Marttica, I.: Risk of Rupture of a Second Aneurysm in Patients with Multiple Aneurysms, J Neurosurg 32:295-299, 1970.

*Dr. Hollin:* Isn't it true, that during the time over which these cases extended the significance of communicating hydrocephalus as a possible cause of the patients' deterioration was probably not as well recognized as today and may have been overlooked?

*Dr. Sencer:* Yes.

*Dr. Hollin:* Dr. Sencer, in your exclusion of surgical cases you stated that 55 of these 59 patients would be considered poor surgical risks. What are your criteria for considering that a patient is inoperable?

*Dr. Sencer:* I would say that patients who, in this particular paper are in Group IV and Group III with active bleeding, are patients that many surgeons would not wish to operate upon, since this factor increases surgical morbidity and mortality.

*Dr. Hollin:* In Case Number 5, for example, the patient was Grade III, semicomatose, and aphasic. Her neurologic state was unchanged for four weeks when she suddenly died from another subarachnoid hemorrhage. We have similar cases that were operated upon after they stabilized. So, I disagree with your Case Number 5 as not being a surgical candidate according to present-day microsurgical criteria. Similarly, in Case Number 7, carotid angiogram revealed a right frontal mass. The patient died with a right frontal intracerebral hematoma. Apparently, you do not believe that surgery may be indicated for evacuation of an acute intracerebral hematoma. Is that correct?

*Dr. Sencer:* I am well aware of the fine surgical results on patients who have intracerebral hematomas; they have been well documented. However, this particular patient who was in coma with an intracerebral mass and a ruptured aneurysm is not exactly the same type of patient that is in the literature. Many patients who have hematomas secondary to head trauma do not have aneurysms. I think that the operative risk of patients with acutely ruptured aneurysms who are critically ill is extremely high; and, I frankly differ with you, in this particular case, as to the indications for surgery.

*Dr. Hollin:* Dr. Sencer, I don't necessarily want to discuss your protocol case by case, but I think it is very important because it brings out a major difference in what we consider to be possible surgical candidates. Let's take Case Number 55, for instance. This patient had headaches, confusion, and left hemiparesis, and he died eleven days after hemorrhage from a posterior communicating artery aneurysm. What was the cause of death in this patient? Was it possible that he had an intracerebral hematoma or hydrocephalus, or did he die of spasm? Similarly, I would like to ask about Case Number 56, in which the patient was stuporous and had left hemiparesis from a temporal lobe hematoma. She died on the third day. Again, we have a question of a patient with an acute intracerebral hematoma secondary to a ruptured aneurysm.

*Dr. Sencer:* The angiogram was probably within hours of his death. I would just state that he probably was in very bad condition after he got back from angiography, in spite of the fact that he had "a mass which might be operated upon."

*Dr. Hollin:* Dr. Sencer, if the possibility exists that some surgeons might operate, you really should not exclude all these cases in order to obtain your low mortality rate. You just arbitrarily eliminated them as not being surgical candidates. I do not understand exactly how you arrive at this.

Let us take Case Number 27. I understand that two neurosurgical opinions were against surgery. The patient had a recurrent hemorrhage on the thirteenth hospital day. I believe, according to present criteria, he would have been a surgical candidate.

*Dr. Sencer:* This case was included predominantly because of a difficult aneurysm in the dominant hemisphere and I respect the opinion of the two other neurosurgeons.

*Dr. Decker:* Dr. Sencer, I would like to bring up the subject of the acute hydrocephalus following subarachnoid hemorrhage. It was frequently not recognized until the last several years. Raimondi reported 32 such cases, 20 undergoing emergency shunting procedures, with 17 improving.* I think that your Case Number 20 would fit the clinical criteria for this diagnosis. Omission of early angiography eliminates confirmation of the clinical impression, and, therefore, therapy. We have only encountered acute hydrocephalus in one case which markedly improved following shunt, but then rebled prior to surgery and died. Three of our patients underwent shunting procedures in the postoperative period; two out of three were known to have moderate hydrocephalus prior to craniotomy. All three cases showed marked and sustained improvement following shunting.

Case Number 51 of Dr. Sencer's series seems to illustrate the point of possible surgery after stabilization despite a fixed or severe focal neurologic deficit. This patient apparently had a subarachnoid hemorrhage with a severe organic mental syndrome and right hemimotor and sensory findings. However, she did improve within the first month of admission before suffering two recurrent sudden subarachnoid hemorrhages which caused her death. We would certainly consider this case for surgery.

In answer to Dr. Sencer's question, xanthochromia does not indicate continuing bleeding; and, therefore, it is not one of our criteria for rejecting a patient for surgery.

It is obvious that Dr. Sencer's group of 55 patients who died consists of dramatically and seriously ill cases compared to some other series. Although the mortality rate of 53 pecent is similar to the national study, with our present knowledge, with possible stabilization, early surgery for intracerebral hematoma, acute hydrocephalus, and treatment with the other modalities, such as hypotensive regimen and antifibrinolysins, we might be able to get the mortality rate for the entire group down to approximately 30 to 40 percent. Dr. Ransohoff recently reported a consecutive series of cases with a 36 percent mortality using initial conservative measures and then surgery.

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* Raimondi, A. J.: Acute Hydrocephalus as a Complication of Subarachnoid Hemorrhage. Paper presented at the American Association of Neurosurgeons Meeting, Houston, Texas, April 1971.

*Dr. Sencer:* I am intrigued by the utilization of the hypotensive techniques, whether it is Slosberg's or otherwise. We know that lowering the pressure may help, as may antifibrinolysin therapy. Medical management can help these patients with subarachnoid hemorrhage due to ruptured aneurysms survive beyond the third or fourth week of their acute illness. At this point they do not need surgery at all, inasmuch as the mortality figures drop so dramatically after the fourth or fifth week.

The critical problem is really the one- to three-week period before the patients stabilize. During this time, many neurosurgeons consider some cases good surgical risks. Once they do get past the fourth or fifth week, the figures for surgical as compared to medical therapy are similar.

I am quite concerned about the management of the patients before the third or fourth week, and I am still not convinced of the efficacy of surgery. More time is needed for evaluating the cases now being treated by surgery under the microscope.

*Dr. Hollin:* Dr. Slosberg mentioned how few late hemorrhages he had and we told him that none of our patients who were discharged subsequently had hemorrhages, with some being followed up to two years. Of your series, how many bled after they left the hospital?

*Dr. Slosberg:* I think it should again be pointed out that the follow-up on patients with hypotensive therapy extends to seventeen years and that late hemorrhages have now been almost completely eliminated.

*Dr. Decker:* Dr. Sencer, could you give us the figures of the mortality and morbidity of the patients at the time of leaving the hospital and the subsequent follow-up.

How about the possibility of chronic or subacute communicating hydrocephalus in this group of patients. It has been reported in the literature, that anywhere from 10 percent to 25 percent of significant hydrocephalus might require shunting. Could you comment on this?

*Dr. Sencer:* As previously noted, we were not as aware of the problem of communicating hydrocephalus in earlier years. Therefore, the cases were not appropriately investigated.

Information on the long-term follow-up of survivors of the initial rupture treated conservatively is not yet available.

*Dr. Hollin:* We were trying to run a series of cases to find out what our overall mortality would be. Therefore, we accepted virtually any case in Grades I to IV, regardless of age limit, for surgery. We wanted to see if our mortality rate would be significantly higher in older patients. As it happens, the only mortalities in the present series were in young patients. However, we do feel that some arbitrary limit should be set. Whether the arbitrary age limit is sixty or sixty-five should be based on various factors, especially the overall, general medical condition. It has been my impression that older patients develop less spasm pre- and postoperatively. In general, if an aneurysm is of favorable size and location, even a patient in the sixties should not be turned down as a surgical candidate. Our anesthesiologists are excellent and the risk of anesthesia per se is low. Previously, this had been a major factor in excluding operations in elderly individuals.

* * *

*Dr. Tuchman:* It has been a fascinating and enjoyable experience to listen to this vigorous interchange. If we are in the Age of Apathy, following the Age of Anxiety, I am delighted that the neurologists and neurosurgeons at The Mount Sinai Hospital have not yet had their fires cooled or their ardors lessened. I have learned much and am sure our readers will, too. Thank you very much gentlemen.

# NEUROLOGIC NOTES

## Frontal Meningioma with an Unusual Clinical Presentation

JOHN LUSINS, M.D.

The occurrence of papilledema in one eye and atrophy in the other is rare. The occurrence of extrapyramidal tremor due to neoplasm is also rare. We present a patient with both findings presumably due to a right frontal meningioma.

### Case Report

Patient was a 32-year-old, right-handed female who was in good health until one year before admission when she noted reduced vision in the right eye. Subsequently, headaches developed in the right side of the head and blurred vision was noted in the left side about two months before admission. One month prior to admission, tremor was noted in the left arm, and later the arm was noted to be clumsy and somewhat slow in movements.

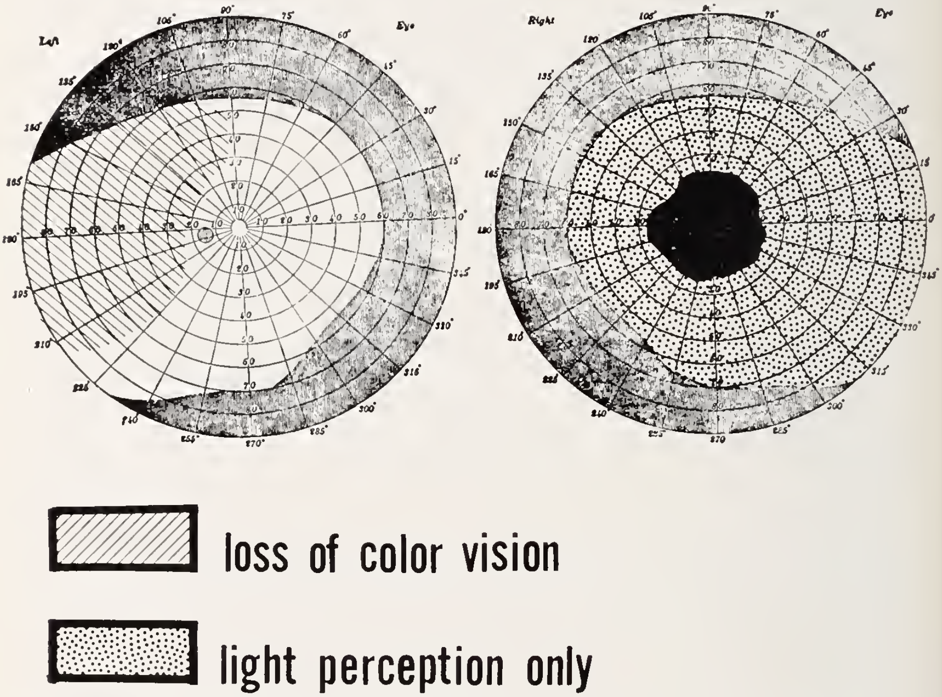
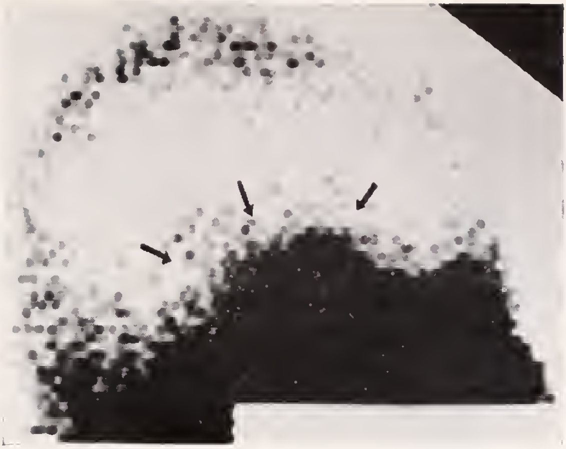


FIG. 1. Visual fields at the time of admission showing temporal loss of color vision on the left and only minimal light perception on the right.

Neurologic examination revealed that the left eye reacted to light, the right eye did not. The fundus on the left showed papilledema without hemorrhages. The right fundus showed definite atrophy. Visual fields revealed minimal light perception in the right eye and a superior temporal defect to color in the left (Fig. 1). The extraocular muscles were normal. There was a decrease in associated movements in the left arm. The deep reflexes were symmetrical throughout and were normal. The tone was increased in the left upper extremity. The left hand tended to be kept with the wrist somewhat semiflexed, the fingers



FIGS. 2 and 3. Brain scan, anterior and lateral views, indicating a large round anterior meningioma.

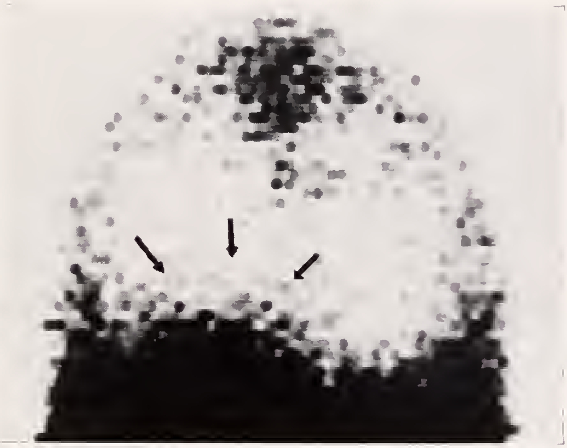


FIG. 3

extended on the metacarpals and the thumb extended and adducted. There was a regular slow tremor of the fingers and thumb of the parkinsonian type. The remainder of the neurologic examination was normal.

The neurologic examination showed that the spinal fluid pressure was 360 mm of water and the protein was 40 mg per cent. No cells were seen. The EEG revealed right cerebral slowing. The brain scan revealed abnormality in the right frontal region (Figs. 2 and 3). Brain angiography revealed a large tumor in the area of the right sphenoid ridge elevating the right frontal lobe and extending as far back as the sella tursica on the right. The appearance of the tumor was typical of meningioma.

## Discussion

Frontal meningiomas which affect the optic nerve can cause optic neuritis and atrophy of that nerve, and later, when the tumor expands, cause papilledema on the opposite side (Fig. 4). This combination of the findings was de-

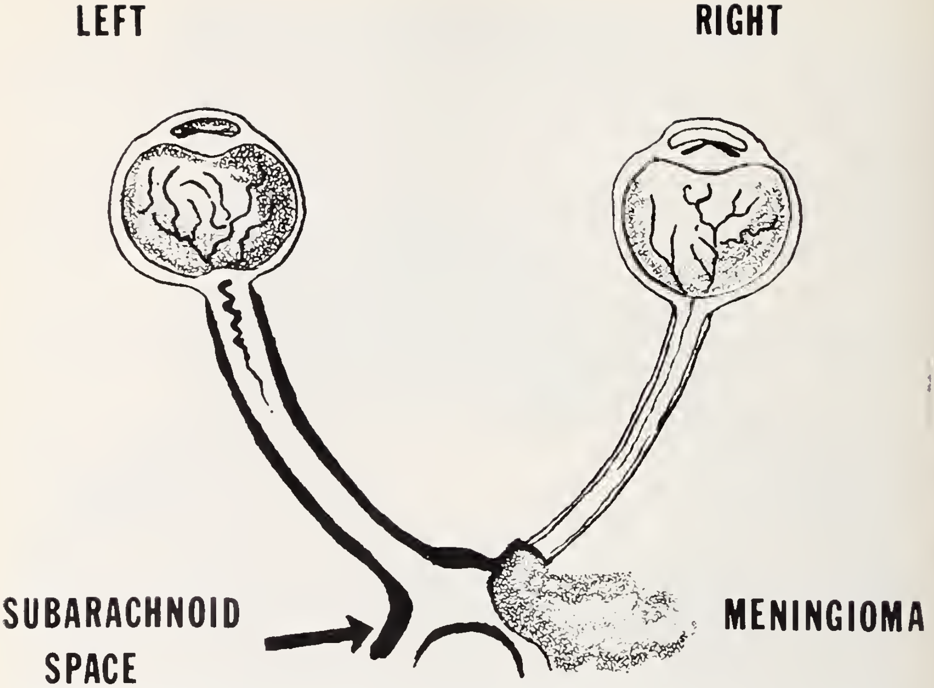


Fig. 4. Diagram showing a meningioma involving the right optic nerve in the area of the chiasm. The meningioma is compressing the nerve and obstructing the subarachnoid space causing optic atrophy on the right. As it expands it causes papilledema in the oposite side.

scribed by Foster Kennedy in 1911 and has remained in the literature under the eponym of Foster Kennedy Syndrome. In the experience of the neurological department of The Mount Sinai Hospital the syndrome is rare, there having been one other case on the service in the last 20 years.

The first reference to a patient with an intracranial, extraparenchymal tumor which was associated with Parkinsonian signs was by Hunt and Lisa in 1927 (2). The tumor was a frontal fibrosarcoma. Nicholson and Turner (3) in a recent review of the literature found a total of 18 cases of intracranial tumors which had associated Parkinsonian signs. Of these, ten were frontal meningiomas. The etiology of the Parkinsonian signs is thought to be secondary to displacement and compression of the basilar ganglion and related structures. In a number of instances these meningiomas were removed with complete regression of these signs and symptoms.

## Summary

We have presented a case of a 32-year-old woman with a right frontal meningioma, papilledema in one eye and atrophy in the other, and right-sided Parkinsonian tremor.

### References

1. Kennedy, F.: Retrobulbar Neuritis as an Exact Diagnostic Sign of Certain Tumors and Abcesses in the Frontal Lobes, Am J Med Science 142:355, 1911.
2. Hunt, E. L., and Lisa, J. R.: Frontal Lobe Tumor: A Case Simulating Epidemic Encephalitis and Parkinson's Syndrome, JAMA 89:1674, 1927.
3. Nicholson, N., and Turner, E. A.: Parkinsonism Produced by Parasagittal Meningiomas, J Neurosurg 21:104, 1964.

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## Addendum

In the paper "Chronic Left Heart Failure—An Experimental Model in the Dog" by Richard P. Lasser, M.D. et al (Mount Sinai J Med 38:440, 1971), reference was not made to two similar cases previously reported:

1. Harthorne et al: Brit Heart J 31:809, 1969.
2. Rose et al: J Thor Surg 62:965, 1971.

Garcia et al (Chest 61:396, 1972) reported a case subsequent to the receipt of Dr. Richard Lasser's manuscript. The Editorial Staff regrets this oversight and is pleased to acknowledge priority of publication by Drs. Harthorne and Rose.

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# The Theory and Function of the Membrane Lung

E. CONVERSE PEIRCE II, M. D.

## Introduction

> "Of the artificial systems, the most attractive is that where, in imitation of the natural pulmonary anatomy, a membrane separates blood from gas."
>
> D. G. Melrose 1959 (1)

Despite the indisputable attractiveness of simulating the natural organ, the clinical acceptance of the membrane lung has been very pedestrian. We have been told for years that blood damage will be less and that longer perfusions will be possible. Brilliant animal investigations have been reported, including those of Kolobow et al who have perfused healthy awake lambs for periods up to 16 days (2), but clinical proof of the effectiveness of the membrane lung has been lacking. Now, suddenly, or at least in a brief span of time, clinical perfusions have been carried out by a number of investigators for periods as long as ten days (3, 4). Many patients have shown improvement during perfusion and several have recovered as the result of the perfusion support.* The membrane lung now enjoys widespread interest and general acceptance seems near at hand.

Today you will hear primarily clinical papers covering the use of the membrane lung both for open heart surgery and for prolonged assisted circulation. You will have an opportunity to see at first hand a number of membrane devices now being manufactured. Most of these are fully disposable. The principal purpose of this paper is to explain the features of the membrane lung by contrasting it both with the natural lung and with nonmembrane devices. I will use this opportunity also to give some of my views on membrane lung testing and the reporting of data.

### *A Comparison of Artificial Lungs with the Natural Lung*

The principal functions of artificial lungs and the natural lung are shown in Table I. The natural lung has a wider range of functions than the artificial devices. Conventional nonmembrane oxygenators fulfill minimum functional requirements including oxygen uptake, carbon dioxide release, and pH regula-

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* As of November 15, 1972, 44 perfusions for respiratory insufficiency have been collected. There have been 7 survivors following perfusions of up to 9.5 days.

tion. The membrane lung, in addition, may be used for blood volume control and is an excellent filter. The list of natural lung functions is not complete and includes cardiac regulation, humidification, and temperature control. Artificial lungs can be adapted for several of these. In addition, all of the lungs may be used for the administration of volatile drugs and their release.

Principal varieties of artificial lungs are shown in Table II. A major classification is based on whether the blood interface is in direct contact with gas, liquid (5), or a membrane. All natural and artificial lungs or oxygenators present a broad interface with blood for oxygen and carbon dioxide exchange. The natural human lung has an area of perhaps 75 square meters (6). With the patient breathing air, the lung can process up to 42 liters of blood per minute with the exchange of as much as 6.2 liters of oxygen (7). Even at these high blood flows, the arterial oxygen tension ($Pa_{O_2}$) is only slightly lower than normal and the arteriovenous oxygen difference can be as high as 14.8 volumes percent. In contrast, the devices we employ for extracorporeal bypass are intended only for near basal blood flows of 5 to 8 liters and oxygen exchange of only 200 to 300 ml per minute when supplied with 100 percent oxygen. Clearly, then, we are talking of devices with a much smaller functional capacity than

TABLE I

*Lung Functions*

| | Natural | Membrane | Nonmembrane |
|---|---|---|---|
| Oxygen Uptake | Yes | Yes | Yes |
| Carbon Dioxide Release | Yes | Yes | Yes |
| Control of pH | Yes | Yes | Yes |
| Blood Volume Control | Yes | Yes | No |
| Filtration | Yes | Yes | No |

TABLE II

*Some Types of Gas Exchange Devices*

Direct Blood to Gas Interface

- Bubble oxygenator
- Disc oxygenator
- Screen oxygenator

Blood—Liquid Interface

Blood—Membrane Interface
- Stationary—No Moving Parts
  - Sandwich
  - Capillary—(Blood or Gas)
- Moveable
  - Rotating
  - Oscillating

the natural lung. The majority of clinical perfusions, moreover, are carried out with gas exchangers that have no separation between blood and gas. Whereas the natural lung serves for a life time, these machines are severely damaging to blood and may be employed safely, judged by our rather crude methods, for only a few hours. In addition, they require constant regulation of blood volume if they are to be safe even for this brief interval. The membrane lungs reduce the blood damage and provide filtration and volume control (Table I) so that they are a major improvement even though they still fall far short of the natural lung in terms of total function.

## Description of the Membrane Lung

### *Blood Damage*

Though they come in many styles, shapes, and sizes, membrane lungs are much more similar than dissimilar. The important line of separation is between devices with a raw blood gas interface and those with a membrane between the two phases (Tables I and II). Lessened blood damage in membrane lungs was anticipated by Clowes in 1956 (8). Lee et al, who did the first definitive research on protein denaturation in gas exchange devices, compared plasma that has been perfused in membrane and nonmembrane devices (9). They showed a variety of physical, chemical, and biologic changes in proteins after 20 hours exposure. These changes were much more marked if there was a blood-to-gas interface. Owens et al showed that exposure of a blood film to a gas resulted in aggregation of neutral fat and that this could cause renal, pulmonary, or cerebral fat embolization in animals and man (10). Confirmtion of increased protein denaturation in nonmembrane devices has come from Dobell et al (11) and Zapol et al (12). Similarly, formed elements are more gently handled in the membrane lung (13). Not only is there less destruction of cells and protein, but potential emboli of protein and cellular debris are quite effectively filtered out by many membrane devices (14). This diminution in blood damage has been confirmed in long-term human perfusions and constitutes the most significant advantage of membrane over nonmembrane lungs (4).

### *Function (Boundary Layer Effects, a Principal Limitation)*

A major developmental problem in membrane lungs has been the construction of a device with sufficiently large oxygen and carbon dioxide exchange. Suitable gas exchange membranes have been too costly and frequently have not been strong enough. These limitations will make the membrane lung expensive for some time. An equally important problem has been the rigorous design requirements for effective oxygen exchange. The transfer of gas in these membrane devices is dependent on the combined resistance of the membrane and the blood phase (Table III) (15). In most devices, the principal resistance for oxygen is in the blood film because oxygen is poorly soluble. Many methods have been tried to decrease this resistance by minimizing the stationary boundary layer

(Table IV) (16–37). In addition, hyperbaria has been employed to increase the oxygen gradient and, consequently, the oxygen exchange for a lung of given membrane area and design (38).

### *Lung Size Required (Dependence on Carbon Dioxide Exchange)*

Although carbon dioxide is about 25 times as soluble as oxygen in blood and more than 5 times as soluble in silicone membranes, the overall function of membrane lungs used for total cardiopulmonary bypass is rather paradoxically

TABLE III

*Approximate Theoretical Resistance to Diffusion in Blood Film for 1 mil Copolymer (MEM-213, 55%) or 3 mil Silicone in a Stationary Membrane Lung*

| Blood Film Microns | $O_2$ Exchange* | % Resistance† | $CO_2$ Exchange‡ | % Resistance† |
|---|---|---|---|---|
| 10 | 262 | 21 | 82 | 5 |
| 50 | 146 | 53 | 69 | 19 |
| 100 | 94 | 71 | 57 | 34 |
| 150 | 69 | 79 | 49 | 43 |
| 200 | 55 | 86 | 43 | 50 |
| 300 | 39 | 88 | 34 | 60 |

* Assuming gas-to-gas diffusion of $O_2 = 330$ ml/M²/min at 37°C, 760 mm Hg, and the diffusion coefficient in blood $= 3.28 \times 10^{-5}$.

† Resistance present in the blood film as percent of total resistance.

‡ Assuming gas-to-gas diffusion of $CO_2 = 86$ ml/M²/min at 37°C, 40 mm Hg, and the diffusion coefficient in blood $= 82 \times 10^{-5}$.

TABLE IV

*Methods of Reducing Boundary Layer Effects in Membrane Lungs*

| General Type | Special Feature |
|---|---|
| Sandwich | Screen in blood phase (16–20) |
| | Pneumatic or hydraulic shim (18, 21) |
| | Multipoint support (22) |
| | Recirculation (23) |
| | Oscillating-gas pressure (24) |
| | Pattern on membrane (25, 26) |
| Coil | Oscillating-gas pressure (27) |
| Capillary | |
| blood inside | Pulsatility (28) |
| blood outside | Interweaving (29) |
| | Pulsatility (30) |
| Envelope | Rhythmic tilting (31, 32) |
| | Vertical cascades (33) |
| Spinning hollow silicone disk | Rapid rotation in blood (34) |
| Toroidal | Rapid oscillation (35–37) |

Numbers in parentheses are references.

limited by carbon dioxide exchange (Table III). This is simply because we have a physiologic limitation of about 40 mm Hg (torr) for the driving pressure of carbon dioxide and it must be lowered even more when respiratory compensation for acidemia is required (Fig. 1). We thus may scale lung size by oxygen need only for partial bypass applications (26).

### *Lung Testing*

Basic performance data should be obtained from gas exchange devices *before* they are used clinically. Of importance are gas exchange, blood trauma, and reliability under a wide range of operating conditions. There is still much misunderstanding and some disagreement regarding the best methods of preclinical testing. A preliminary statement has been formulated by a standards subcommittee of the American Society for Artificial Internal Organs (39).

### *In Vitro versus Ex Vivo Testing*

Salzman has presented an excellent analysis of advantages and disadvantages of various modes of device testing (Table V) (40). For extracorporeal devices, such as the membrane lung, we are limited to in vitro and ex vivo modes. To obtain all of the information needed, we must utilize both approaches.

Basic performance data for membrane devices are best obtained not with scale models but with final production models in vitro, where accurate control of blood flow, gas flow, temperature, and other independent variables is possible (21–23). Data may be secured over a wider range and a steadier state than is usually feasible with an ex vivo perfusion where an animal is in the circuit (41).

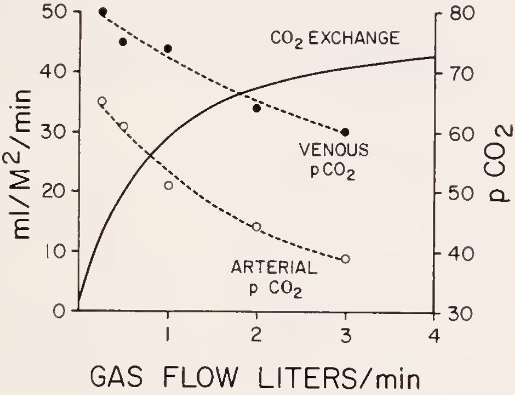

FIG. 1. As in a normal mammalian lung, carbon dioxide exchange in the membrane lung varies directly with minute volume. This means that the device can be utilized for $P_{CO_2}$ and pH regulation. The venous and arterial $P_{CO_2}$ data were obtained in a total cardiopulmonary bypass experiment. As oxygen exchange is less influenced by minute volume than is the case with the natural lung, alkalemia and low arterial $P_{CO_2}$ values may be readily corrected by reducing gas flow. If the artificial lung size is chosen so that maximum gas flow is not required for the elimination of predicted carbon dioxide production, acidemia and high $P_{CO_2}$ values may be corrected by increasing the gas flow. Reproduced by permission of Appleton-Century-Crofts, New York, *Surgery Annual 1972,* Edited by P. Cooper and L. Nyhus.

TABLE V

*Modes of Device Testing*

| Advantages | Problems |
|---|---|
| In Vitro | |
| Best standardized | Artifacts easily occur |
| Easiest to study in detail | May not be relevant |
| Ex Vivo | |
| Subject-device interaction* | Tends to be insensitive |
| | Time limitations* |
| | Subject interaction* |
| | Conditions tend to change with time* |
| | Activation of substances |
| | Exhaustion of substances* |
| | Range of testing limited* |
| In Vivo | |
| Subject-device interaction* | Large experimental errors |
| | Discontinuous observations |
| | Few data points |
| | Expensive |
| | Can't study transients easily |

* Added for completeness.

Data on oxygen and carbon dioxide exchange, perfusion pressure, blood volume, and compliance of the device will be reliable. If the experiments are complete and carefully conducted, additional information of this nature will not ordinarily be obtained when the devices are subsequently used for animals and humans. An important feature of careful in vitro testing of function is the ease with which the effects of modifications may be detected (15, 21, 23). The omission of this step has, I believe, on occasion led manufacturers inadvertently to the dangerous modification of devices in active clinical use. Such surprises should rarely occur.

It has been argued that tests need not be carried out with blood because results of gas-to-gas exchange (Table VI) and gas-to-water exchange are accurately predictive (41). Useful results are certainly obtained with these tests, especially gas-to-gas studies, and they probably have a place in device development in a sophisticated laboratory. I feel certain, however, that general use of water instead of blood would lead to inferior data and greatly increase the danger of error. The use of dye curves with water perfusion similarly provides interesting data that, nevertheless, is not immediately interpretable in terms of clinical function (Fig. 2).

It may further be argued that such things as clotting and fibrin build-up within the device that modify function will only be evident if these tests are performed ex vivo (42). Surely, ex vivo tests must be performed to study various applications and biologic interactions but this need not be done over the entire range of function. It should also be pointed out that time-dependent changes occurring during ex vivo tests with animals such as sheep may be quite different than those found in human use (42). Blood coagulation systems of sheep are

TABLE VI

*Gas to Gas Exchange in the Membrane Lung*

| Lung | $O_2$* | $CO_2$† | Material | Effective Thickness mils |
|---|---|---|---|---|
| Travenol (STX 290) | 91 ± 6 | 26 ± 2 | Silicone | 11.8 |
| Bramson (2 cell) | 104 ± 2 | 30 ± 1 | Silicone (reinforced) | 10.2 |
| G.E.-Peirce | 162 ± 13 | 44 ± 2 | Copolymer (MEM-213, 55%) | 2.0‡ |
| Landé-Edwards | 214 ± 11 | 55 ± 3 | Silicone | 5.0 |
| Abcor Q | 237 ± 29 | 62 ± 3 | Silicone | 4.5 |
| DOW (GE Series) | 305 ± 20 | 94 ± 6 | Silicone (capillary) | 3.5 |
| (SRO Series) | 375 ± 28 | 108 ± 7 | | 2.9 |

* $ml/M^2/min$ (760 mm Hg).

† $ml/M^2/min$ (40 mm Hg).

‡ Equivalent to about 6 mils silicone.

Figures recomputed from data of Galletti et al (41). Gas data ± SD. Effective membrane thickness estimated from gas exchange (see Table III for predicted gas-to-gas exchange for 3 mil silicone).

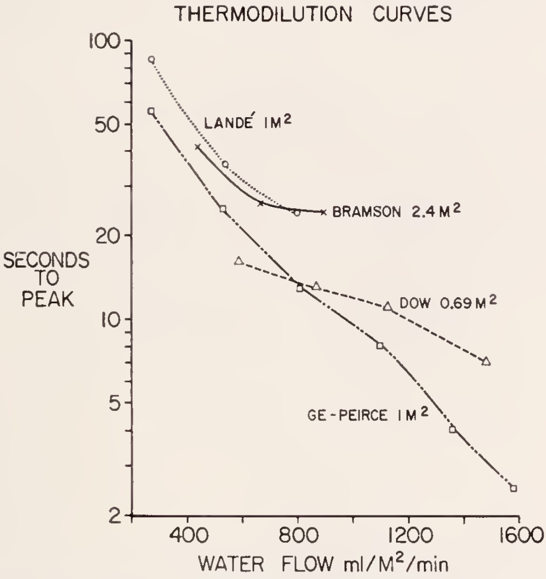

FIG. 2. The flow distribution characteristics of four membrane devices examined by means of thermodilution curves using water perfusion were reported by Galletti et al (41). Data are here presented as time to curve peak in seconds versus water flow in $ml/M^2/min$. Such information can be used to study changes in blood flow distribution at different flow rates and may be very helpful at the stage of lung design. These data are, however, difficult to interpret and do not supplant actual measurement of gas exchange in blood. They may, in fact, be rather misleading. Though producing quite different results in this test, the Landé, Bramson, and G.E.-Peirce devices all perform well in terms of gas exchange and clinically.

very different from the human. For example, fibrinolysis is minimal. In addition, red cell fragility, as shown by a shear stress test, is very different (Table VII) (43). Animal data should, therefore, not be given excessive weight in making clinical decisions.

### *Reporting Gas Exchange Data*

Since data are inversely useful to the multiplicity of methods used in reporting, the desirability of standardization is evident. Some modification of Galletti's "rated flow" method would appear to be desirable (44). The "rated flow" is the flow at which blood of a 40% packed cell volume enters the device 65% saturated and leaves 95% saturated. "Rated oxygen transfer" is the oxygen uptake in ml/$M^2$/min at "rated flow" of blood and gas. "Rated gas flow" is the flow beyond which no measurable increase in carbon dioxide transfer occurs with a constant blood input $P_{CO_2}$. Some modifications of the numbers would be advisable but the operational concept is sound and easy to understand. It is to be hoped that agreement can be reached at a suitable national or international conference.

### *Blood Damage*

The most important single factor in blood damage is probably interaction between blood and the inner surfaces of the extracorporeal system (wall interaction) (45). For this reason it is necessary to keep in mind the sequence of events at the blood solid interface (Table VIII). The changes are probably not

TABLE VII

*Red Blood Cell*

Jet Fragility Test 25°C

| | Index | Range | Number |
|---|---|---|---|
| Human | | | |
| Male | 1.00 | (0.88–1.14) | (26) |
| Female | 0.89 | (0.78–1.04) | (12) |
| Fetus | 0.73 | (0.68–0.78) | (11) |
| Dog | 1.06 | | |
| Cat | 1.98 | | |
| Mouse | 1.56 | | |
| Duck | 1.67 | | |
| Chicken | 2.70 | | |
| Sheep | 2.88 | | |
| Goat | 6.70 | | |

Numbers above 1.00 indicate that cells are less easily hemolyzed by shear stress than are human red cells. Data of Forstrom (43).

TABLE VIII

*Sequence of Events at the Blood-Solid Interface*

1. Formation of a protein monolayer that may be denatured. The character of the layer may change with time.
2. Reversible or irreversible platelet adhesion.
3. Red and white blood cell adhesion.
4. Fibrin deposition.
5. Fibrin breakdown.

very different qualitatively whether heparin is used or not. Nevertheless, for our purposes in studying artificial external organ systems, the blood should be fresh, heparinized, and preferably human. Citrated blood, because of a low level of free calcium ions, "may lead to erroneous conclusions concerning the passivity of the surface in question" (46). As noted above, blood from different animals may differ markedly and this may complicate interpretation and comparison of work by different investigators. Hemodynamic factors must also be considered and are more important than previously thought (Fig. 3) (47).

In the case of blood trauma, some real problems arise both with in vitro and animal ex vivo testing (Table IX). Although in vitro testing is very valuable for the comparison of devices (13) and for the detection of special problems (Figs. 3 and 4), the results may differ markedly from those obtained when there is interaction between the lung device and the subject being perfused.

In the case of red blood cell damage there may be actual hemolysis which is readily quantitated in vitro by measuring the plasma hemoglobin (Fig. 5) but there may be equally or more important subhemolytic (sublethal or occult) damage (45, 48) which may be characterized only by special methods (Table X). Unfortunately the tests for sublethal or occult damage provide too few data points, are not standardized, or are difficult to interpret. In consequence, these tests have not been useful for the comparison of one device with another. The problem is further complicated ex vivo because the plasma hemoglobin rise is moderated by its removal from the blood. Furthermore, many sublethally damaged cells are destroyed by the reticuloendothelial system without any corresponding rise in plasma hemoglobin (49). This removal of sublethally damaged cells is slow and may take hours or days. This phenomonon may be determined currently only by measuring the red blood cell half life or the excess carbon

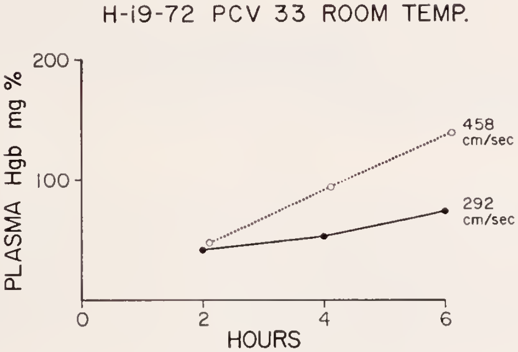

FIG. 3. The effect of a shear strain produced by a low speed jet in a paired standard trauma test perfusion at room temperature is illustrated (47). Data from a circuit without a jet are shown in figure 5. A Robicsek orifice plate flow meter was used to produce the jet. This introduced minimal additional wall interaction. Fresh heparinized human blood was perfused at 500 ml/min for an estimated 500 circulations in six hours. Even at low mean jet velocities, there was substantially more bulk hemolysis than occurs when a G.E.-Peirce lung was used at approximately "rated flow" (1000 ml/M$^2$/min). These findings emphasize the importance of hemodynamic factors in blood damage and are particularly pertinent because the critical velocity for damage has previously been reported by Forstrom to be higher than 1500 centimeters per second (43). Reproduced from Trans Am Soc Artif Int Organs 18:342, 1972 with permission.

TABLE IX

*Blood Damage Studies*

| | In Vitro* | Ex Vivo† |
|---|---|---|
| Red Blood Cells | Progressive rise in plasma hemoglobin with time‡¶ | Variable pattern of plasma hemoglobin change with time§¶ |
| White Blood Cells | Small change in numbers¶ | Large phasic change in numbers§¶ |
| Platelets | Small change in numbers¶ | Large drop in numbers§¶ |
| Lipoproteins | Detectable changes‖ | Detectable changes§‖ |
| Fibrinogen | Changes not usually seen | Postperfusion increase§ |
| Clotting factors | Changes not usually seen | Changes not usually seen |

* Results vary with animal species, temperature, lipid level, etc. but good controls are possible.

† Important species variations occur (see text).

‡ Devices, circuit variations, etc. usefully compared.

§ Device comparison difficult because of biologic variability.

‖ Standardized tests not available.

¶ Important but variable changes in morphology and function.

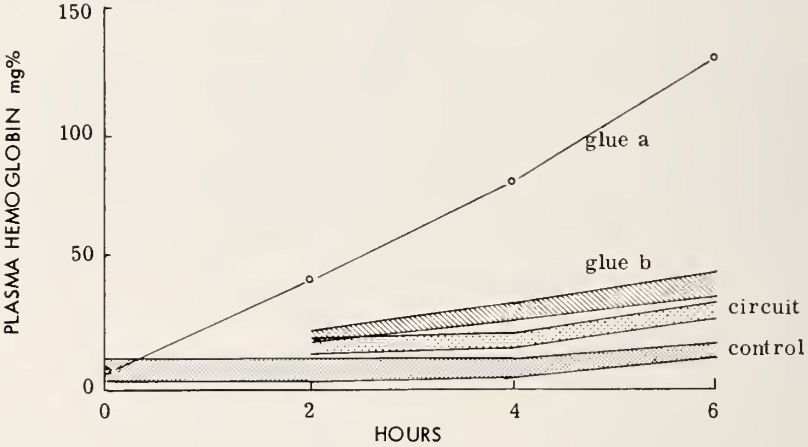

FIG. 4. Blood damage may occur as a result of chemical interaction as well as from surface contact and from hemodynamic strains. During the preclinical development of the G.E.-Peirce lung, high hemolysis values were found when lungs using glue a were tested in a paired standard trauma test using dog blood at room temperature (13). A change to glue b eliminated the problem and confirmed the role of glue a in the high hemolysis levels. A residuum of ethylene oxide after sterilization may cause similar difficulties in perfusion devices. It is easy to see why damage studies need to be performed with final sterile production models of devices before they are used in humans.

monoxide production from heme catabolism (49). Since occult damage may be very much greater than actual hemolysis during perfusion, its magnitude is clearly of importance in determining the suitability of clinical devices. The suggestion of Bernstein et al (50) that the multiplier factor may be as large as 500 (i.e., occult damage 500 times as important as immediate hemolysis) would appear to be unduly pessimistic at least as far as membrane devices are con-

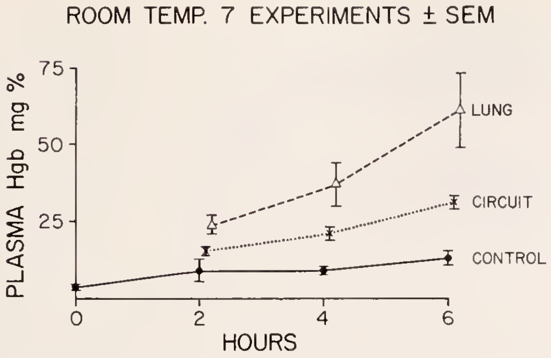

FIG. 5. Results of paired trauma studies using human blood are illustrated for the G.E.-Peirce lung. Six hours represents approximately 500 transits or recirculations of the entire test blood volume. The control value is for blood kept at room temperature but not circulated. Passage through the circuit approximately doubles the control plasma hemoglobin level and the use of the lung approximately doubles it again. It is thought that most of the red blood cell damage in the lung represents wall contact with the membrane but some may be due to hemodynamic strains in the ports. The test provides a means of testing modifications in membrane, porting, sterilization and so forth. Reproduced with permission from Trans Am Soc Artif Int Organs 18:342, 1972.

TABLE X

*Sublethal Red Cell Damage*

| Some Possible Tests |
|---|
| *In Vitro* |
| Changes in morphology* |
| Sodium pump integrity* |
| Metabolism—aerobic and anaerobic* |
| Autohemolysis† |
| *Ex Vivo and In Vivo* |
| CO production from heme catabolism* |
| Changes in osmotic fragility† |
| Cell survival‡ |

* Test not standardized

† Interpretation not clear.

‡ Each animal provides a single data point.

cerned. Data presently available indicate that lethal damage does not exceed the rate of normal red blood cell death and that plasma hemoglobin levels do not rise astronomically during long-term perfusion. A significant factor is probably passivation of the membranes and other portions of the extracorporeal circuit by proteins (51) so that red cell damage and plasma hemoglobin levels decrease with time (52).

In the case of white blood cells perfused in vitro there is not a very big absolute drop in the count, but important morphologic and functional changes can be shown. Kusserow et al, for example, have shown a diminution of oxygen consumption and a lower phagocytic activity (53). In ex vivo perfusions, the situation is quite different (Fig. 6; Table IX). Cells are rapidly removed in the perfused subject by mechanisms not yet fully elucidated and there is a very rapid fall in the white blood cell count. The white blood cells, however, are rapidly replenished, especially the neutrophiles. In a perfusion of several hours or longer the white cell count frequently reaches a higher level than in the beginning, with a preponderance of the cells being young forms (52).

A somewhat similar situation occurs in the case of platelets. Perfused in vitro, there is only a modest drop in numbers although again functional changes may be detected (Fig. 7). In an ex vivo perfusion, there is a rapid drop in the number of platelets indicating that those marked by the circuit are removed in some manner. Platelets are not replenished as rapidly as white blood cells and the platelet level remains depressed throughout the perfusion (Fig. 6) (52, 54). In some long-term human perfusions platelets have been decreased at the start and during the perfusion have been depressed to levels that are dangerously low. Some investigators have utilized platelet transfusions but their efficacy has not been demonstrated (55).

## Summary

The membrane lung differs from conventional oxygenators by virtue of the membrane interposed between the blood and gas phases. This makes possible a reduction in blood trauma, simplified blood volume control, and obviates the need for a separate filter. To determine the relative effectiveness of different

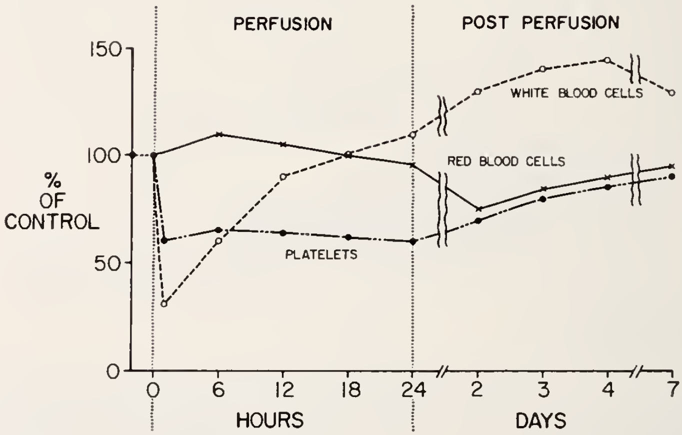

Fig. 6. Change in the numbers of formed elements of the blood produced by ex vivo perfusion are diagramatically represented. Red blood cell levels increase somewhat initially as cells stored in the spleens, etc. are released. There is then a small progressive decline due to actual hemolysis and due to the nonhemolytic retirement of cells occultly damaged in the circuit. Postperfusion, red blood cells continue to be withdrawn because of occult damage. This is followed by a slow restorative process. Both platelets and white blood cells show a rapid decline in numbers immediately after the start of perfusion. In the case of platelets, there is then a relative stabilization of numbers with a slow postperfusion return toward normal. In the case of the white blood cells, there is a rapid return toward normal, and frequently to a higher level than normal, and there is usually a postperfusion leukocytosis. This rapid white cell replenishment represents the release (and probably accelerated production) of more juvenile forms than were present initially. These ex vivo changes are very different from those occurring in an in vitro perfusion (see text).

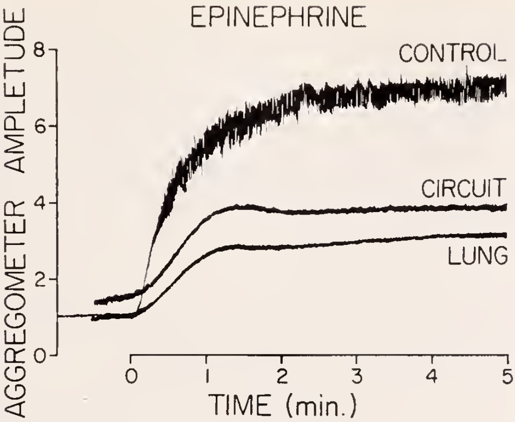

Fig. 7. Platelet function was measured after six hours of an in vitro test perfusion using the degree of aggregation induced with epinephrine, collagen, or adenosine triphosphate. The effect of epinephrine (illustrated) is typical. Platelets that are not perfused (control) show rapid aggregation, platelets from circuit blood (no lung) show diminished aggregation, while those from the lung circuit show the least aggregation.

lungs, real data are needed—first from controlled in vitro and ex vivo laboratory experiments, second from prolonged perfusions in animals, and finally from actual clinical cases. To be useful, these data must be disseminated in a reasonably uniform manner. Several devices have now been subjected to this whole sequence of testing. They appear to fulfill their earlier expectations and have been shown to be suitable for standard clinical use and for long-term perfusions of at least ten days. We will hear today some of the specific experiences with the four currently available membrane lungs. Several others are almost as far along and can be seen in the exhibits.

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# Regulation of Coagulation during Prolonged Perfusions Using Membrane Oxygenation

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## Introduction

With the advent of disposable membrane oxygenators, long-term perfusion for ventilatory and/or cardiac support has become a reality (1–4).

Reports in the literature vary as to the amount of trauma to blood components and/or clotting disorders caused by extracorporeal perfusion and whether such trauma is caused by the pump or by the method of effecting gas exchange (5–7). This paper reports changes observed during eight clinical perfusions (one short-term and seven prolonged). Prevention of coagulation depletion deficiencies consequent to volume expansion and selection of a simple, accurate method of determining heparin dosage are emphasized.

## Method

Volume expansion and replacement was carried out, as much as possible, by using packed red blood cells and fresh frozen plasma (to replace factors V and VIII, known to be deficient in bank blood). Platelet transfusions were given, when available, for platelet counts below 30,000 or for clinically significant wound-site bleeding. Salt-poor albumin was used to promote urine flow and avoid edema. Dextran was eschewed at all times (except as a preterminal event in Case 3).

Anticoagulation was followed by means of plasma partial thromboplastin times (PTT) (activated in Cases 2 through 8; normal range, 35 to 45 seconds; not activated in Case 1; normal, less than 100 seconds). Partial thromboplastin time tests were performed on citrated plasma, using the Clotek System® (Hyland). Platelin® (Warner-Chilcott) was used in the nonactivated PTT tests in Case 1.

The site of blood sampling was from the central venous pressure line when possible, to ensure that clotting tests reflected the coagulation status of the patient, not of the pump (8). Approximately 500 cc of blood was drawn each day for various tests; this amount was included in the intake and output record.

Blood used for transfusion was unreconstituted ACD (9) (except in Case 1, in which 2500 units of heparin was added to each 500 ml). All blood was warmed prior to transfusion, using various combinations of a Hemokinethotherm® (Dupaco) blood warmer, a Brown-Harrison type heat exchanger within the pump circuitry, and hypo-hyperthermia blankets.

A 25 to 40 micron mesh Barrier Extracorporeal Blood Filter® (Johnson &

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Johnson) was used for all blood to be transfused, whether via pump or patient vessel. A blood filter was included within the pump circuitry only in Case 1.

A roller pump with proportional weigher for automatic control (Olson) and two 3 $M^2$ Landé-Edwards oxygenators were used in all cases. Blood flow was venoarterial and averaged 1.5 to 3.0 L/min; and oxygen flow rate was 12 to 15 L/min. All membranes were prerinsed with five to six bottles of 25 percent salt-poor albumin (12.5 gm each), after the initial rinsing for the sodium bicarbonate or sodium chloride used to prevent sticking of the membranes. In addition to membrane lung support, Cases 7 and 8 underwent renal dialysis during perfusion; and Case 3 had a Kantrowitz intraaortic balloon in place throughout bypass.

Clinical assessment of the patient's coagulation status relied primarily on sequential fibrinogen levels (10) and platelet counts, with the PTT used to gauge heparinization. Although not all tests were performed on all patients, additional tests performed included prothrombin time (PT), using Simplastin® (Warner-Chilcott) (normal expressed against a control); thrombin time (TT) (11), using bovine thrombin (normal expressed against a control), with and without protamine titration; whole-clot lysis time; euglobulin lysis time (12) (normal, less than 120 minutes); quality of clot retraction; plasminogen levels (normal, 2.0 to 5.0 casein units); amount of red-cell "fall-out"; direct and indirect Coombs' tests; and tests for the presence of fibrin split products (FSP) (normally absent) using FI-Test® reagent (Hyland). The validity of this test was cross-checked by a staphylococcus clumping test. Serum calcium levels were performed as a part of the automated screening profile done in the Biochemistry Department, using an SMA 12. Plasma hemoglobin levels were performed by a modification of the benzidine method (13) (normal, 0.2 to 2 mg %). Serum haptoglobin levels (normal, usually 200 mg %) were estimated semiquantitatively using electrophoresis (the Hyland Kit). Antibody studies using fibrinogen and platelet proteins were performed on membranes obtained from a Landé-Edwards oxygenator used in Case 4.

## Results

The eight cases are briefly summarized in Table I. Selected laboratory data and daily volume expansion are shown in Figures 1, 2, and 4 (Cases 1, 4, and 8); PTT values and heparin doses are shown in Figure 3 (Case 4) and Figures 5, 6, and 7 (Case 8). Changes in clotting tests during perfusion for open heart surgery are shown in Table II.

Changes in clotting tests followed the same pattern in all cases; fibrinogen levels and platelet counts approximately halved with the onset of perfusion. No significant clotting abnormalities were detected in control samples (except in Case 8). With heparinization there was consistent elevation of PT, TT, and PTT values. No reproducible pattern was found in the scattered abnormal whole-clot lysis times, euglobin lysis times, or amount of red-cell fall-out. Clot retraction was impaired in virtually all samples after institution of bypass and

## TABLE I

*Summary of Eight Cases Studied on Membrane Oxygenator*

| Case | Duration bypass | Drop in Platelet Counts | Drop in Fibrinogens | Rise in Bilirubins |
|---|---|---|---|---|
| 1) 34 yr. old ♀ Thoracotomy - Chondrosarcoma | 60 hrs. (unconscious) | 252.0 → 48.0 | 770 → 350 | 0.75 → 7.12 → 4.00 |
| 2) 52 yr. old ♂ Mitral value | 2.5 hrs. (anesthetized) | 249.0 → 65.0 | 350 → 145 | ? |
| 3) 77 yr. old ♀ Acute M. I. | 29.5 hrs. (unconscious to conscious) | 217.0 → 130.0<br>↓<br>17.0 | ? → 295<br>↓<br>90 | ? |
| 4) 27 yr. old ♂ Gun-shot wound | 38.5 hrs. (unconscious to semi-conscious) | 116.0 → 68.0<br>↓<br>34.0 | ? → 682 | ? → 6.65 |
| 5) 18 yr. old ♂ Bronchopneumonia (Hodgkin's) | 54 hrs. (unconscious to conscious) | 248.0 → 145.0 | 1560 → 920 | 2.30 → 10.20 → 6.06 |
| 6) 35 yr. old ♂ Bleomycin toxicity (Hodgkin's) | 16 hrs. (semi-conscious) | 53.0 → 18.0 | 1390<br>↓<br>642 → 145<br>↓<br>325 | 4.45 → 4.00 |
| 7) 58 yr. old ♀ Epiderm Ca Lung Thoracotomy | 40 hrs. (unconscious to conscious) | 276.0 → 29.0 | 520 → 215 | 0.50 → 5.61 |
| 8) 62 yr. old ♂ Flu | 150 hrs. (conscious) | (144.0) → 75.0 | 530 → 245 | 0.60 → 19.15 |

## TABLE II

*Hematologic Data during Membrane Oxygenation for Open Heart Surgery, Case 2*

| (A.F.) | 2 days pre-op | After induction anesthesia | "Pump blood" | 15 mins. on bypass | 1 hour 45 mins. on bypass | Off bypass |
|---|---|---|---|---|---|---|
| Platelet Count (250cells per ml.) | 259.0 | 249.0 | – | 65.0 | 163.0 | 179.0 |
| Fibrinogen (350 mg%) | 471 | 350 | 74.5 | 145 | 225 | 194 |
| Plasminogen(2.0→5.5 caseinolytic units) | 3.0 | 3.4 | 0.6 | 1.34 | 1.90 | 2.0 |
| Euglobulin Lysis Time (>120 mins.) | >90' | >120' | >120' | >120' | 60' | 60' |
| Thrombin Time (secs.) | "Normal" | 24.5"<br>(c. 20.0) | – | >120<br>(c. 22.4) | >120<br>(c. 22.4) | >120<br>(c. 22.4) |
| Prothrombin Time (secs.) | 19.2" | 12.4" | >120" | >60" | >60" | 52.2" |
| PTT (secs.) | – | 44.8"<br>(c. 38.4) | >120"<br>(c. 38.4) | >120"<br>(c. 38.4) | >120"<br>(c. 38.5) | >120"<br>(c. 38.5) |
| Factor Ⅴ (% normal) | 88% | 90% | <10% | <10% | 11% | 15% |
| Factor Ⅷ (% normal) | ≅ 100% | – | – | – | – | – |

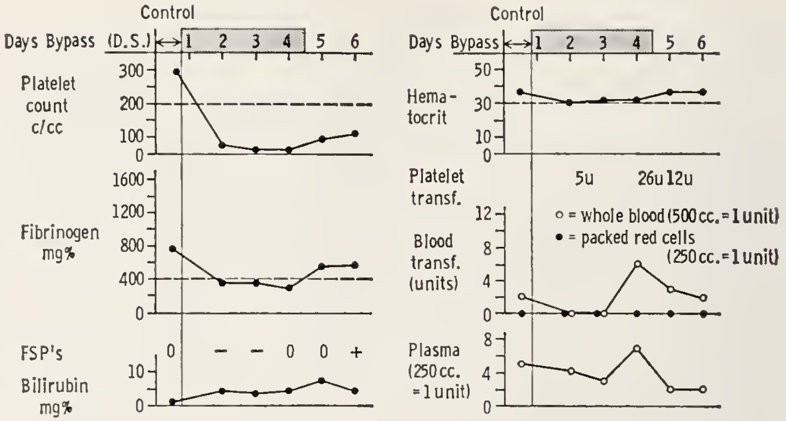

Fig. 1. Major changes in hematologic laboratory data related to daily blood component therapy during 60-hour partial bypass for respiratory insufficiency, Case 1.

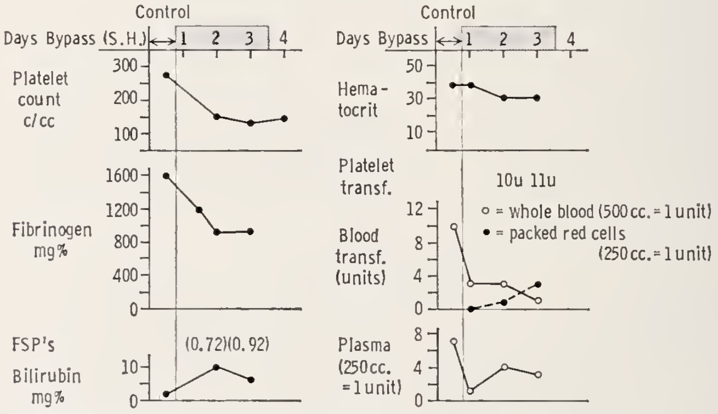

Fig. 2. Major changes in hematologic laboratory data related to daily blood component therapy during 38½-hour partial bypass for respiratory insufficiency, Case 4.

plasminogen levels were low. Fibrin split products were absent during perfusion, with the exception of Case 8, in whom they were present prior to bypass, and throughout the hospital course; the staphylococcus clumping test showed 32 micrograms of fibrin degradation products per milliliter (normal, less than 4 $\mu$g/ml) when FI-Test reagent gave a positive result at a serum dilution of 1:32. No clots were found in any of the blood filters. Serum calcium levels were not significantly disturbed. Blood cultures were negative throughout. Due to the complexity of these cases and the multiplicity of laboratory results, emphasis has been placed on presentation of data from four cases.

## Case Reports

*Case 1.* A 34-year-old woman developed progressive respiratory insufficiency after thoracotomy for excision of metastatic chondrosarcoma: the primary lesion had been excised from the left humerus 4½ years earlier. Partial bypass was begun on the third postoperative

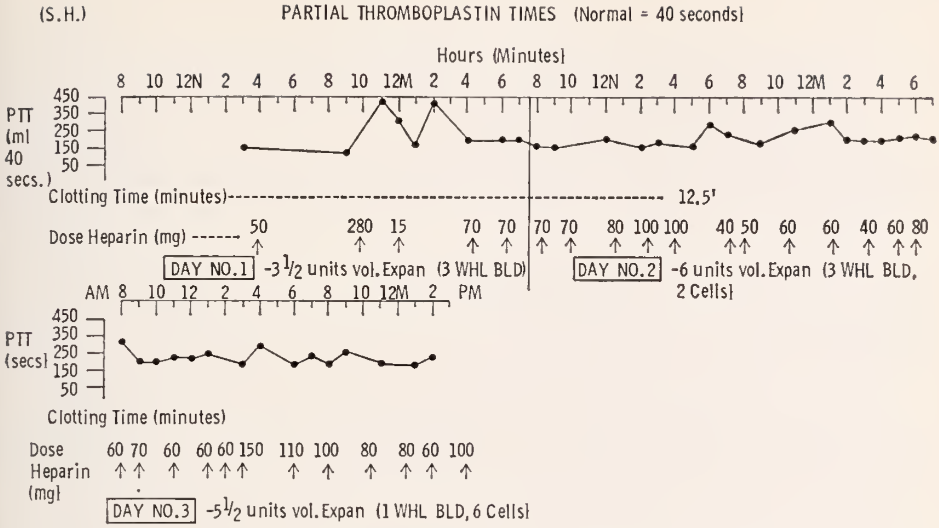

Fig. 3. Heparin dosages as related to partial thromboplastin times and daily blood component therapy, Case 4.

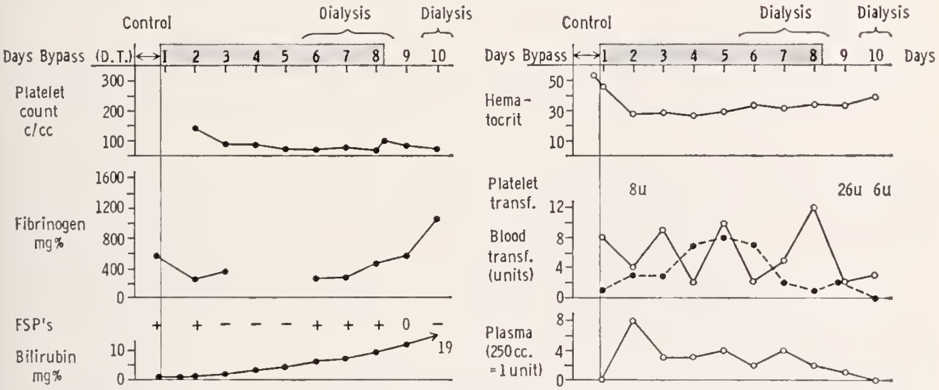

Fig. 4. Major changes in hematologic laboratory data related to daily blood component therapy during 150-hour partial bypass for respiratory insufficiency, Case 8.

day (14). Hemorrhage occurred after 50 hours of bypass due to the combined effect of high levels of anticoagulation and thrombocytopenia (at that time, PTT was 445 seconds; platelet count was 21,000). A major factor in the high level of anticoagulation attained in this case was the addition of heparin to each unit of blood prior to transfusion to reconstitute it. Hemorrhage was controlled before weaning from bypass (at 60 hours) by omission of further heparin and administration of a large platelet transfusion.

In addition to diffuse anoxic neuronal degeneration and diffuse hyaline membrane pneumonia, autopsy revealed fibrin thrombi in alveolar capillaries and small arteries of the lung, as well as in the renal glomeruli. Bilateral hippocampal and gastrointestinal hemorrhages were also noted.

*Case 2.* Changes in clotting tests (Fig. 2) can be attributed to institution of bypass alone, bypass being undertaken only for the two and one-half hours necessary for mitral valve re-

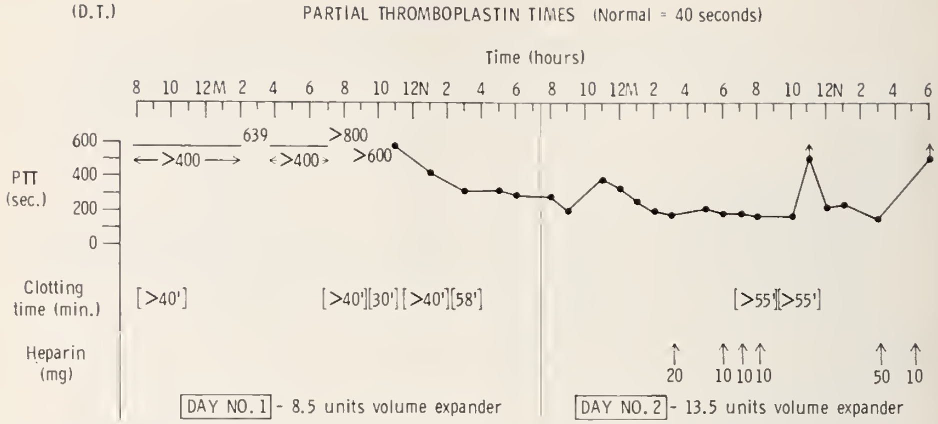

Fig. 5. Heparin dosages as related to partial thromboplastin times and daily blood component therapy. Case 8, days 1 and 2.

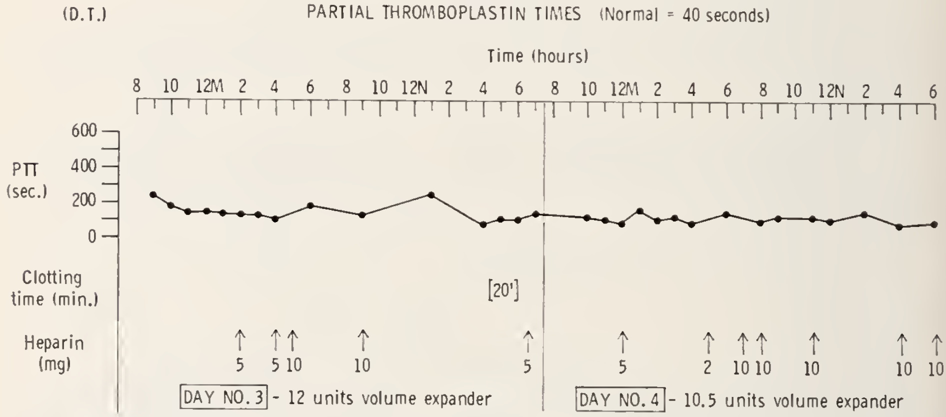

Fig. 6. Heparin dosages as related to partial thromboplastin times and daily blood component therapy, Case 8, days 3 and 4.

placement. The circuit in this case was primed with 4 units of whole blood and 500 cc of Normosol® (Abbott). No excessive bleeding was encountered during surgery or during the uneventful postoperative course.

*Case 4.* Progressive respiratory insufficiency followed thoracotomy for resection of metastatic osteogenic sarcoma in this 18-year-old man. The primary lesion had been excised from the right leg one year before; and metastases had been removed at two previous thoracotomies. Partial bypass was begun three days postoperatively.

Management adhered closely to the regimen previously outlined and there was no evidence of clinical bleeding during this perfusion. The cause of death was occluding tumor thrombus extending from the pulmonary vein to the mitral valve.

Autopsy revealed confluent, panlobar pneumonia with hyaline membrane formation and intraalveolar hemorrhage. No other signs of hemorrhage or thrombosis were found. Residual sarcoma was present.

Antibody studies showed adherence both of fibrinogen and platelet proteins, coating the blood surfaces of the used membranes.

The plasma homoglobin level doubled after fifteen minutes of bypass, and then stabilized

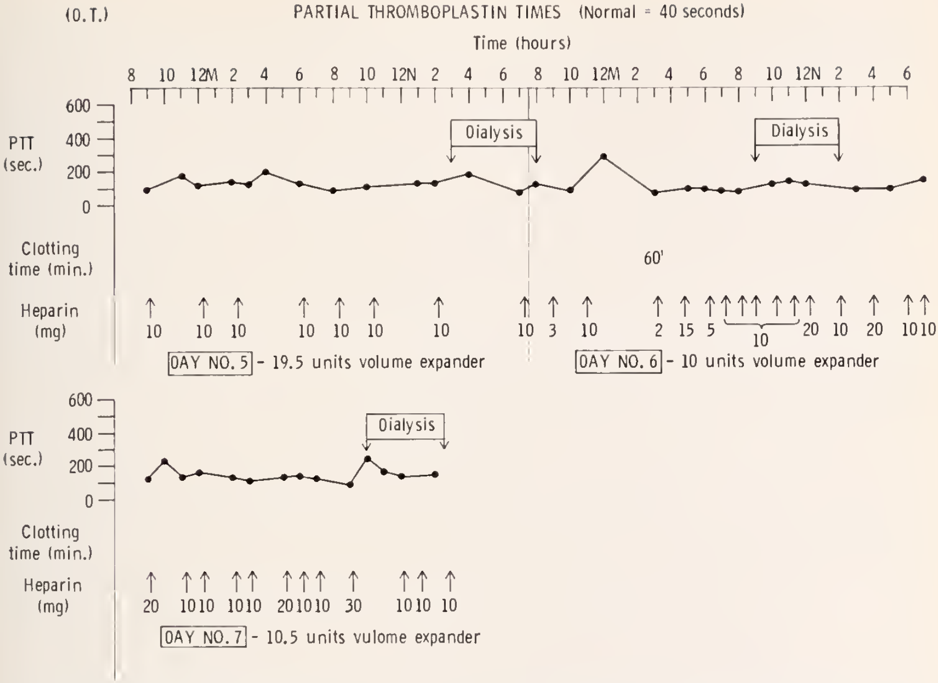

FIG. 7. Heparin dosages as related to partial thromboplastin times and daily blood component therapy, Case 8, days 5, 6, and 7.

within the normal range. The haptoglobin level was 100 mg %, despite hemolysis, just prior to institution of bypass and fell to 0 mg %, with a larger total amount of hemoglobin-haptoglobin complex noted, after 30 hours of bypass. Serum bilirubin values are shown in Figure 2. Direct and indirect Coombs' tests remained negative.

*Case 8.* This 62-year-old man was admitted from another hospital with progressive respiratory insufficiency secondary to interstitial pneumonitis. He was placed on partial bypass immediately. The course was complicated by persistent bleeding associated with initial placement of intravascular catheters in femoral vessels which had antecedent peripheral vascular disease. Two fasciotomies were subsequently required to relieve the pressure caused by hemorrhage into the leg, which was never controlled. Very high creatinine phosphokinase suggested muscle breakdown. Urine myoglobin was then measured and ranged from 277 to 1088 mg/L before renal dialysis (normal, less than 4 mg/L). These high levels of myoglobin were reflected in the serum bilirubin levels recorded in Figure 4. Despite 150 hours of support, the respiratory status did not improve sufficiently and the patient expired two days after cessation of bypass. As previously mentioned, this patient had a consumption coagulopathy on admission (prior to bypass), with fibrin split products present in the control blood sample.

Autopsy restricted to lung biopsy only revealed interstitial viral pneumonia with recent hemorrhage, pulmonary edema, and hyaline membrane formation.

## Discussion

Adequate evaluation of heparinization during extracorporeal bypass has assumed a new urgency with the advent of prolonged bypasses for respiratory and/or cardiac insufficiency. It is no longer possible to complete bypass within the few hours required to perform open-heart surgery. A practical program for determining both the frequency and the dosage of heparin is necessary. Avoidance of concomitant coagulation disorders is, obviously, an inseparable part of any such regimen, because most forms of timed clotting tests (the tests used clinically to assess heparinization) are altered by deficiencies of other clotting components. Avoidance of depletion deficiencies will also reduce wound site bleeding that results from the synergistic effects of multiple minor deficiencies.

The whole blood clotting time (WBCT) has not proved accurate or sensitive enough for this task. This test is widely recognized as unreliable and one which reflects deficiencies in many aspects of the coagulation mechanism. Equally undesirable is the time necessary to obtain a result (many minutes to hours). Responsive adjustment of heparin dosage is therefore impossible using the WBCT (15) and one is forced to employ excessive doses of heparin in order to ensure that some degree of heparin effect is always present.

The PTT test appears to be much more satisfactory (16–17). In the cases reported in this paper, the PTT was usually performed once an hour, permitting frequent adjustment of heparin dosage. The consistent, initial halving of fibrinogen levels and platelet counts noted in all cases in this series was not prevented by the standard initial large dosages of heparin (for example, 2 mg/Kg body weight). The high level of anticoagulation maintained throughout Case 1 did not prevent the progressive thrombocytopenia that seemed to be a major precipitating cause of hemorrhage. Subsequent patients have been given doses of heparin sufficient to prolong the PTT to three to four times the normal (120 to 160 seconds) without any increased tendency to hemorrhage or thrombosis. The WBCT (glass) was usually within the normal range (5 to 15 minutes).

The importance of serial determinations in evaluating the status of the patient is most obvious when considering the prebypass fibrinogen levels; control samples ranged from 350 to 1,560 mg % (normal, 300 to 400 mg %). This hyperfibrinogenemia cannot be attributed to the disease shared by most of our patient population, since some of the patients without cancer also had high initial fibrinogen levels. Sepsis may be the cause. The necessity for baseline values, if the sharp decrease in fibrinogen is not to be over looked, deserves emphasis (18).

Since it is difficult to attribute the abrupt falls in fibrinogen and platelets to anything other than some form of consumptive process, a brief discussion of present concepts of consumption coagulopathy seems in order at this time.

It has been increasingly apparent that the classic criteria for establishing the diagnosis of consumption coagulopathy are in need of modification if better clinical results are to be obtained. The situation is analogous to the problem of diagnosing septic shock. By the time hypotension and metabolic acidosis develop, much less a positive blood culture, the mortality rate approaches 80 percent. This contrasts sharply with the nearly 100 percent survival if therapy is begun during the early respiratory alkalosis stage, as described by MacLean (19).

The proliferation of more sensitive tests for detection of fibrin split products is illustrative of the recognition of this need for earlier diagnosis of consumption coagulopathy (20–24). Since most of these tests are available only at scattered centers and on a limited basis, they remain, at least at this time, of little benefit to the clinician confronted with a coagulation problem.

We have, therefore, adopted as criteria for the diagnosis of early, or incipient, consumption coagulopathy the progressive, simultaneous decrease both in fibrinogen level and platelet count. No attempt has been made to differentiate a "hyperthrombotic" (25) from a "hypercoagulable" state, since the therapy for both is similar. The criteria have seemed reasonable because these simultaneous decreases in fibrinogen and platelets cannot otherwise be ascribed to any single clotting disorder. They are out of all proportion to any so-called dilutional effects of volume expansion, particularly those attributed to bank blood, which has normal fibrinogen levels. Furthermore, the institution of low dose heparin (20 to 40 mg IV every 4 hours) has promptly reversed this downward trend in other patients who were not on extracorporeal bypass.

Elevation of thrombin times (part of the classic criteria for a consumption) would not be expected until a significant (and hence, detectable) amount of fibrin split products was present, since it is these same split products that are believed to be the cause of the prolongation in thrombin time.

The low plasminogen levels (reflecting activation of the fibrinolytic system) reported in this paper tend to corroborate that a form of consumption is the cause of initial depletion of clotting components seen during extracorporeal bypass. Subsequent appearance of fibrin split products appears to confirm this concept.

Since antibody studies showed adherence of both platelet and fibrinogen proteins to the blood surfaces of membrane sheets used during bypass, the hypothesis of an initial, relatively self-limiting consumption coagulopathy seems reasonable. This concept provides a coherent explanation for the changes in coagulation component levels reported in this paper. This extracorporeal consumption would largely cease after the exposed membrane surfaces had been coated during initial exposure to the blood (25).

Epsilon aminocaproic acid was avoided because it is a dangerous agent in the presence of a consumption coagulopathy. Thrombosis after EACA therapy for fibrinolysis, assumed to be unaccompanied by a hypercoagulable state, has been amply documented in the literature (26–32).

Hemolysis attributable to bypass, as estimated by plasma hemoglobin levels, has been insignificant. Though haptoglobin levels fell to zero, plasma hemoglobin levels never exceeded normal. The possible contribution of intravenous albumin concentrates to the clearance (33) of free hemoglobin is not clear, since methemalbuminemia was not noted (though sensitive testing to detect its presence was not performed).

## Summary

Neither gas exchange nor hemolysis have proved troublesome during seven long-term, partial bypass procedures using membrane oxygenation.

Maintenance of hemostasis was achieved using a regimen employing fresh frozen plasma and platelet transfusions in conjunction with careful regulation of anticoagulation by frequently performed partial thromboplastin times.

Lower levels of anticoagulation than have usually been considered necessary have proved perfectly feasible. Whole blood clotting times were maintained largely within normal range, while partial thromboplastin times were three to four times normal, with no evidence of gross or microscopic hemorrhage or thrombosis.

No heparin and/or calcium reconstitution of ACD blood for transfusion was necessary.

An initial, relatively self-limited, consumption of clotting factors occurred with initiation of bypass. This is attributed, at least partly, to coating of the membrane surfaces upon exposure to blood.

### Acknowledgments

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# Comparison in Children of the Peirce-General Electric Membrane Oxygenator with a Bubble Oxygenator

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## Introduction

The majority of open heart operations today are performed with a variety of oxygenators that utilize a blood-gas interface for gas exchange. This blood-gas interface produces many undesirable changes such as red blood cell damage, alterations in coagulation factors, denaturation of fat and protein, and pulmonary damage. These changes make perfusions longer than five or six hours unsafe (1, 2).

Membrane oxygenators interpose a semipermeable membrane between the blood and gas phases and moderate the undesirable effects of the blood-gas interface. Osborn, using the Bramsen membrane lung, and Baffes and Carlson, using the Landé-Edwards membrane lung, have reported successful open heart surgery with these oxygenators (3–5). During their perfusions oxygenation was excellent without carbon dioxide retention and there was minimal destruction of the formed elements of the blood.

Peirce, in conjunction with the General Electric Company, has developed a new membrane oxygenator utilizing a copolymer of silicone and polycarbonate (2, 6). This report presents a two-and-one-half year experience with the use of the Peirce G-E membrane oxygenator in children undergoing open heart surgery as well as a comparison with a similar group of patients undergoing perfusion using a Temptrol® bubble oxygenator (Bentley).

### *Clinical Experience*

Over a two-and-one-half year period from November 1969 through March 1972 thirty-six children underwent total cardiopulmonary bypass with the Peirce G-E membrane oxygenator combined with a Sarns roller pump. The various cardiac conditions for which the membrane oxygenator was used are listed in Table I. The age of the patients varied from 3 to 65 months (mean 27) and 13 children were less than twelve months old. The body weights ranged from 2.6 to 17 Kg (mean 9.5) and ten children weighed less than 5 Kg (Table II). Serum hemoglobin, a coagulation survey, renal function studies, arterial blood gases, and serum potassium were determined prior to, at the completion of, and twenty-four hours following cardiopulmonary bypass. During perfusion arterial and venous blood gas values were obtained at fifteen minute intervals.

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In three infants pulmonary function studies were measured preoperatively and twenty-four hours postoperatively.

The 1 $M^2$ oxygenator was used for children who weighed 5 Kg or less, the 2 $M^2$ for those of 5 to 14 Kg, and the 3 $M^2$ for those of 15 Kg or more. Priming volume was 120 ml per $M^2$. For the entire system, 2500 ml was required. During perfusion an attempt was made to maintain high perfusion flow rates within the limits determined by the venous return. In all patients 100 percent oxygen was used for oxygenation with the oxygen flow being 3L/min/1000 cc of blood. Moderate hypothermia (30°C) was used for those patients in whom the aorta was cross-clamped for ten minutes or more.

The same metabolic, hematologic, pulmonary, and renal determinations were made on a randomly selected but not entirely comparable group of pediatric patients who underwent perfusion with a pediatric Temptrol disposable blood oxygenator combined with a Sarns roller pump. The various cardiac conditions for which this oxygenator was used are listed in Table I. In this group of patients oxygenation was maintained with a gas mixture of 97 percent oxygen and three percent carbon dioxide at flow rates of 3L/min/1000 cc of blood. The total priming volume averaged 2000 cc of whole blood.

## Membrane Oxygenator

The longest perfusion lasted 180 minutes and the shortest 36 minutes, with an average duration of 99 minutes. In each operation the membrane lung functioned adequately and leaks did not occur. The oxygenating capacity of the membrane lung was more than satisfactory. During perfusion the mean arterial $Po_2$ averaged 420 mm Hg (range, 54 to 670) (Table III). It was rarely below 100 mm Hg (two patients) and was above 300 mm Hg in twenty-five patients. With improvements in design and manufacture of the membrane lung, there was no further difficulty obtaining adequate oxygenation at satisfactory flow

TABLE I

*Cardiac Conditions and Type of Oxygenator*

| | Membrane Oxygenator | Bubble Oxygenator |
|---|---|---|
| Ventricular septal defect | 7 | 8 |
| Tetralogy | 9 | 6 |
| Transposition—great vessels | 8 | 2 |
| Atrio-ventricular canal | | 2 |
| Total anomalous pulmonary venous return | 3 | |
| Atrial & ventricular septal defect | 1 | |
| Truncus arteriosus | 1 | |
| Anomalous origin—left coronary artery | 1 | |
| Atrial septal defect & PDA | 1 | |
| Double outlet—right ventricle | 2 | |
| Sinus Valsalva—right ventricular fistula | 1 | |
| Cardiomyopathy | 1 | |
| Atrial septal defect | 1 | |

TABLE II

*Age, Weight, and Type of Oxygenator*

| | Membrane Oxygenator | Bubble Oxygenator |
|---|---|---|
| Weight in Kg | | |
| 5 or less | 10 | — |
| 6–10 | 9 | 3 |
| 11–20 | 16 | 15 |
| >20 | 1 | |
| Age in months | | |
| 1–12 | 13 | 1 |
| 13–24 | 9 | 5 |
| 25–48 | 12 | 12 |
| >48 | 2 | |

TABLE III

*Flow Rates and Blood Gases during Perfusion*

| | Membrane | Bubble |
|---|---|---|
| *ml/Kg/min* | | |
| Flow rates | 127 (50–250) | 129 (55–250) |
| Arterial $\text{Po}_2$ mm Hg | 420 (54–670) | 352 (70–640) |
| Arterial $\text{Pco}_2$ mmHg | 31.5 (18–51) | 35 (20–50) |
| pH | 7.38 (7.25–7.59) | 7.38 (7.21–7.53) |

rates as occurred with the membranes used earlier. Carbon dioxide exchange was also adequate. During the perfusions the $\text{Pco}_2$ of the arterial blood ranged from 18 to 51 mm Hg (mean, 31.5). Venous $\text{Pco}_2$ was measured in 22 patients and in only three patients was it above 45 mm Hg. The mean venous $\text{Pco}_2$ for the entire group was 38 mm Hg. Blood flow rates during perfusion ranged from a minimum of 80 ml/Kg to a maximum of 250 ml/Kg (mean, 127). This was adequate for the patients' needs since metabolic acidosis was rarely a problem during any of the perfusions and the mean pH during perfusion was 7.30 (range, 7.25 to 7.59) (Table III).

At the end of bypass arterial blood samples invariably showed excellent oxygen saturation (mean, 99 percent saturation) with a $\text{Po}_2$ ranging from 72 to 612 mm Hg (mean, 253), $\text{Pco}_2$ ranging from 26 to 54 mm Hg (mean, 34.3) and pH between 7.31 and 7.59 (mean, 7.45) (Table III).

Minimal destruction of the formed elements of the blood occurred during the perfusions. Plasma hemoglobin rose from a control value of 14.1 mg percent to 69.1 mg percent immediately following cardiopulmonary bypass (Table IV). These changes were believed to be secondary to the use of intracardiac suction and not due to the oxygenator itself since the highest values of hemoglobin were obtained in those patients in which there was excessive use of the intracardiac suction system. Twenty-four hours postoperatively, the plasma hemoglobin had

TABLE IV

*Plasma Hemoglobin and Platelets during Perfusion*

| | Membrane | Bubble |
|---|---|---|
| Plasma Hgb mg% | | |
| Control | 14.1 (2–40) | 12.5 (1–35) |
| End Perfusion | 69.1 (10–140) | 87.0 (22–120) |
| 24 hours | 22.0 (2–90) | 34.5 (4–92) |
| Platelets per mm$^3$ × $10^{-3}$ | | |
| Control | 238 (70–335) | 217 (35–360) |
| End Perfusion | 121 (50–300) | 98 (20–235) |
| 24 hours | 129 (50–285) | 107 (15–200) |

Mean Value and Range

decreased to 22 mg percent. The mean platelet count averaged 238,000 preoperatively, decreased to 121,000 at the conclusion of the perfusion, and increased to 129,000 twenty-four hours postoperatively (Table IV). No abnormalities were detected in the prothrombin time, the thrombin time, or the partial thromboplastin time. Ten patients had evidence of circulating fibrinolysins and were treated with epsilon aminocaproic acid (Amicar®). The decrease in platelet count also reflected damage to the platelets from the use of intracardiac suction. In spite of the fall in platelet count there was no excessive bleeding postoperatively in any of the patients due to coagulation defects. Two patients were reoperated upon the night of the operation because of bleeding, and in each patient a specific cause for the bleeding was found. One patient developed acute renal failure in conjunction with a low cardiac output following a difficult correction of tetralogy of Fallot. Otherwise, postoperative measurements of renal function and serum potassium were within normal limits. Blood urea nitrogen (BUN) levels ranged from 11 to 33 mg percent (mean 18.3) postoperatively. Urine output during the first twenty-four hours was at least 0.5 ml Kg/hr except for the patient who developed acute renal failure.

Pulmonary function studies were carried out on three infants preoperatively and one day postoperatively. There was no change in tidal volume, dynamic compliance, or alveolar ventilation from preoperative values. There was an increase in functional residual capacity and decrease in minute ventilation. Because of the small number of patients studied, final conclusions regarding the effects of perfusion on pulmonary function cannot be made (Table V).

There were five postoperative deaths. None of the deaths appeared to be directly or indirectly related to a deficiency of the oxygenator. Two patients died as a result of pulmonary hypertension despite complete correction of the cardiac lesion. An infant with cardiomyopathy, who was operated upon with a mistaken diagnosis of atrial and ventricular septal defects, died as a result of low cardiac output. A four-year-old child exsanguinated from an infected suture line one month postoperatively. The other death occurred two months

TABLE V

*Pulmonary Function Studies before and after Perfusion*

| | $V_t$ ml/Kg | FRC ml/Kg | MV L/Kg/min | Compliance ml/cm $H_2O$/Kg | Resistance cm $H_2O$/L/sec/Kg | Alveolar Ventilation L/Kg/min |
|---|---|---|---|---|---|---|
| Membrane | | | | | | |
| preop (N = 3) | 16.3 | 26.5 | .74 | 3.6* | 5.4 | .23 |
| 1 day postop (N = 3) | 16.4 | 46.0 | .57 | 3.4* | 7.1 | .30 |
| Bubble | | | | | | |
| preop (N = 8) | 7.6 | 41.8 | .36 | 1.25 | 4.1 | .22 |
| 1 day postop (N = 8) | 9.5 | 44.2 | .42 | 3.7 | 6.6 | .22 |

* data from 2 patients

postoperatively from unknown causes after successful correction of total anomalous venous return.

### Bubble Oxygenator

The 18 patients perfused using the bubble oxygenator were somewhat older (39.5 vs. 27 months), heavier (mean, 14 vs. 9.5 Kg), and had shorter periods of perfusion (mean, 76 vs. 99 min) (Table II). The values of the physiologic variables measured during and after bypass are shown in Table III. Arterial blood samples during bypass showed excellent oxygenation; the mean $Po_2$ was 352 mm Hg (range 70 to 640). There was adequate carbon dioxide exchange and the $Pco_2$ ranged from 20 mm Hg to 50 mm Hg (mean 35). There was generally the normal acid-base balance with pH values varying from 7.21 to 7.53 (mean, 7.38). The plasma hemoglobin averaged 12.5 mg percent preoperatively, increased to 87 mg percent at the completion of the perfusion and decreased to 34.5 mg percent twenty-four hours postoperatively. The platelet count preoperatively averaged 217,000 and decreased to 98,000 at the conclusion of the perfusion with an increase to 107,000 twenty-four hours postoperatively. No abnormalities were detected in the prothrombin time, the thrombin time, or the partial thromboplastin time. Six patients had evidence of circulating fibrinolysins which were treated successfully with epsilon aminocaproic acid (Amicar). There was no excessive bleeding postoperatively in this group of patients. Postoperative determinations of BUN and serum potassium were within normal limits.

Pulmonary function studies were obtained in eight patients preoperatively and one day postoperatively. There was no change in tidal volume and a slight lowering of the functional residual capacity. The minute ventilation increased by 16 percent over the preoperative value by the first postoperative day. Compliance increased three-fold and there was no change in alveolar ventilation. These changes in pulmonary function are similar to those reported previously in studies on patients undergoing thoracotomy alone or cardiopulmonary bypass (5, 7) (Table V). There was one postoperative death related to incomplete repair of a complicated lesion.

## Discussion

Our study has shown the Peirce-General Electric copolymer membrane lung is capable of providing adequate gas exchange at satisfatory flow rates during total cardiopulmonary bypass in children. Its oxygenating efficiency and carbon dioxide elimination were comparable to that of the disposable Temptrol bubble oxygenator. Although mean plasma Hgb values were lower and platelet counts higher after perfusion with the membrane lung, these differences were not statistically significant. Two factors may be responsible for this. First, due to the nature of the cardiac lesions, considerable intracardiac suction was required. Second, the lesions were repaired within a reasonably short period of perfusion. There is a linear relationship between the duration of perfusion, the amount of intracardiac suction and the amount of blood destruction. A greater difference in the amount of blood destruction between the two oxygenators might have been noticed if less cardiotomy suction and longer perfusions had been required.

There was no difference in tidal volume, functional residual capacity, minute ventilation, compliance, airway resistance, or alveolar ventilation in studies performed one day postoperatively between the two groups. These changes in pulmonary function are similar to studies reported previously in patients undergoing thoracotomy alone or cardiopulmonary bypass (5). There was no obvious impairment in the postoperative status of the central nervous system in any of these patients.

In this clinical trial, the Peirce-General Electric copolymer membrane lung has been shown to be an effective, reliable and safe oxygenator for total cardiopulmonary bypass of relatively short duration. Further studies are needed to determine its usefulness in the repair of complex cardiac lesions where prolonged perfusion will be necessary to carry out complete correction or in long-term circulatory or pulmonary assistance.

## Summary

The new Peirce-General Electric copolymer membrane oxygenator combined with a Sarns roller pump was used clinically for the first time on 36 children undergoing open heart operations. The children ranged in age from 3 to 65 months (mean, 27) and weighed between 2.6 and 17 Kg (mean, 9.5). The priming volume of the oxygenator averaged 120 ml of blood per square meter of membrane. Perfusion varied from 36 to 180 minutes (mean, 99) depending upon the complexity of repair. During perfusion, flow rates averaged 127 ml/Kg with a $P_{O_2}$ average of 420 mm Hg (range 54 to 670). There was no significant change in acid-base balance during or immediately after the perfusion. Serum hemoglobin averaged 14.1 mg percent preoperatively, increased to 69.1 mg percent at the completion of the perfusion, and decreased to 22 mg percent by 24 hours postoperatively. Platelet count preoperatively averaged 238,000, decreased to 121,000 at the conclusion of the perfusion, and was 129,000 one day postoperatively. There was no excessive bleeding postoperatively due to coagulation defects. Roughly comparable results were found in 18 bubble oxygenator

perfusions though patients were older (39.5 vs. 27 months), heavier (14 vs. 9.5 Kg), and were perfused for a shorter time (76 vs. 99 min).

The copolymer membrane has been shown in this group of patients to be an effective, reliable and safe oxygenator for total cardiopulmonary bypass. Although plasma hemoglobin values were lower and platelet counts higher after membrane oxygenator perfusion than when the bubble oxygenator was employed, the differences were not statistically significant.

## Acknowledgments

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# Landé-Edwards Membrane Oxygenator for Heart Surgery: Method and Oxygen Transfer

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Kolff noted that blood exiting his artificial membrane kidney became oxygenated. Utilizing this observation, he developed a membrane oxygenator which avoided a direct air-blood interface. Cumbersome but effective membrane oxygenators were used clinically by Kolff in 1956 (1).

Prototypes of a new membrane oxygenator were developed by Landé and associates in 1967 (2). Blood was separated from gas by a thin silicone rubber sheet folded and separated by grooved plastic wafers. Blood and gas were manifolded into multiple countercurrent flow paths 3 cm in length. Further models of the membrane oxygenator were developed.

The present 3 $M^2$ disposable Landé-Edwards Membrane Oxygenator (Model 5110-3) (Edwards Laboratories) was first used clinically in 1969 for emergency prolonged cardiopulmonary bypass (3, 4). The crucial difference between the membrane and filming or bubbling devices appears to be the absence of direct contact between blood and gas which results in fewer physical and chemical stresses on blood in the membrane oxygenator (5).

This communication describes the present method of total heart-lung support with the Landé-Edwards Membrane Oxygenator and oxygen transfer in the first 100 patients.

## Present Method

*Circuitry:* The first twenty-three patients were linked to a recirculation circuit with a pump before and after the membrane (6). This complicated recirculation method was found to be unnecessary.

The presently preferred method allows drainage of venous blood by gravity flow directly into the membrane oxygenators as first described by Baffes (7) and modified by us (Fig. 1). Two 3 $M^2$ Landé-Edwards Membrane Oxygenators are connected in parallel for patients weighing under 80 Kg (three units for patients over 80 Kg).

In the gravity drainage circuitry, venous blood flows from the level of the operating table through the membrane oxygenators into the arterial reservoir which is placed slightly higher than the oxygenators to prevent air embolism across the membranes. The reservoir is 30 to 50 cm below table height to provide sufficient pressure head for adequate blood flow. An arterial pump removes blood from the reservoir and returns it to the patient's arterial line via a heat exchanger. Blood collected from the left ventricle as well as the cardiotomy suctioned blood is introduced ahead of the oxygenators, via a cardiotomy

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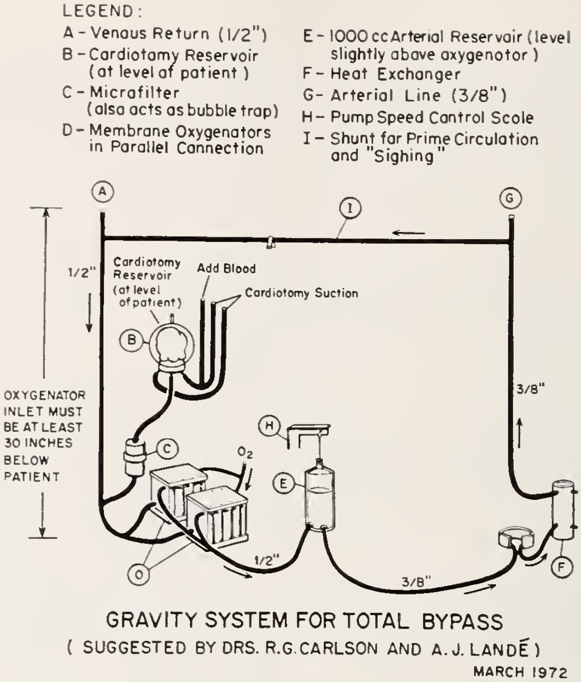
LEGEND:
A - Venous Return (1/2")
B - Cardiotomy Reservoir (at level of patient)
C - Microfilter (also acts as bubble trap)
D - Membrane Oxygenators in Parallel Connection
E - 1000 cc Arterial Reservoir (level slightly above oxygenator)
F - Heat Exchanger
G - Arterial Line (3/8")
H - Pump Speed Control Scale
I - Shunt for Prime Circulation and "Sighing"
A
I
G
1/2"
Cardiotomy Reservoir (at level of patient)
Add Blood
Cardiotomy Suction
B
OXYGENATOR INLET MUST BE AT LEAST 30 INCHES BELOW PATIENT
3/8"
H
C
O2
E
D
1/2"
3/8"
F
GRAVITY SYSTEM FOR TOTAL BYPASS
( SUGGESTED BY DRS. R.G.CARLSON AND A.J. LANDÉ )
MARCH 1972

FIG. 1. Two (or three) 3 $M^2$ Landé-Edwards Membrane Oxygenators are linked for venous blood to flow by gravity drainage through the membranes into an arterial reservoir. Blood from the cardiotomy suction line drains into the venous line via a cardiotomy reservoir and a 40 $\mu$ microfilter. A standard roller pump returns the blood to the patient's artery. (The earlier methods of recirculation, or of gravity flow with the cardiotomy suctioned blood directly entering the arterial reservoir have been discontinued).

reservoir and filter. The screen filter with 40-micron diameter pores removes tiny clots, fat debris, and other particulate matter from the blood (8) (Barrier Extracorporeal Blood Filter, Johnson & Johnson). Presterilized assembled circuitry consisting of tubing, connectors, and arterial reservoir are used (Olson).

*Priming:* The circuit is primed with 5% dextrose in water, serum albumin, and buffered bottled blood. One liter of 5% dextrose in water is added to the system for each 3 $M^2$ oxygenator. This dissolves the sodium chloride dusted on the membrane during manufacture to prevent the silicone rubber membranes from sticking to one another. The sodium concentration is measured whenever hemodilution is used. Approximately normal to twice normal saline (0.9 to 1.8 gm%) results. Normal saline or other crystalloid fluid is added to completely fill the system. All air is removed by rapidly rotating the pump while alternately clamping off oxygenators and leaving only one unclamped. Next, 12.5 gm of salt-poor serum albumin per oxygenator is added to coat the membranes and other plastic parts in the circuit to reduce adhesive trauma to platelets and other formed elements. Finally, buffered bottled blood is added via the cardiotomy suction reservoir, displacing the priming fluid with care not to admit any air in the process. Significant hemodilution is avoided for long pump runs.

*Flow Technique:* Flow of oxygen and blood is similar to bubble oxygenator methods during hypothermia. Dry oxygen is transmitted into $O_2$ inlets at five liters per minute per oxygenator. Maximum blood flow rates are sought and are usually 3000 to 4500 ml/min. (40 to 60 ml/Kg/min) Arterial blood pressure is maintained above 60 mmHg and the hematocrit is kept between 30 to 35 percent by adding blood or Ringer's lactate solution when needed.

Pulmonary ventilation and spontaneous cardiac contractions are continued during cooling to 30°C and during rewarming when practical. Ventilating the lungs supplements membrane oxygen and carbon dioxide exchange during partial cardiac support.

Periodic high flow perfusion every 60 minutes through each membrane oxygenator unit with the entire venous output appears to avoid "membrane atelectasis" by reopening any unused portions of the oxygenator. Twice in our early experience the membrane oxygenator circuit was filled with saline and kept without gas flow for three days prior to operation. Diffusion of the stagnant saline through the membrane caused "oxygenator edema" and poor gas exchange. This indicated the necessity of drying the oxygen passages when prepriming was utilized.

*Patient Selection:* Patient selection initially was limited to the more complicated cases requiring multiple aorto-coronary artery vein grafts, tedious epicardial dissections after previous mammary artery implantations, and/or other complex structural repairs, or to patients with low cardiac output requiring cannulation and cardiopulmonary support prior to opening the thorax and beginning the cardiac operation (6). Later, as its effectiveness became apparent, the membrane oxygenator was used for patients requiring routine open heart operations for single coronary artery grafts, or congenital heart or acquired valve disease.

*Oxygen Transfer:* Specimens for blood gases and hematocrit determinations were drawn at half hour intervals during total heart-lung perfusion. Samples were obtained simultaneously from the mixed venous inlet line and the arterial line immediately beyond the oxygenator outlets.

The determination of pH, $P_{O_2}$, $P_{CO_2}$ were performed at 37°C with an IL-113 blood gas analyser (Instrument Laboratories). Oxygen saturations were calculated using the Siggaard Anderson nomogram with corrections being made for pH, temperature, and base excess or deficit (9). Oxygen transfer was calculated by the formula:

$O_2$ transfer ml/min =

$$\left[\left(\frac{\mathrm{Hct}}{3}\right)(1.34)(\mathrm{A} - \mathrm{V}\,\%\mathrm{O_2Sat}) + (0.003)(\mathrm{P_{aO_2}} - \mathrm{P_{vO_2}})\right]\frac{\text{Blood flow ml/min}}{100}$$

The rectal temperature was considered to be the core temperature during cooling and rewarming although a steady state was never present.

## Results

Previous communications described the relative safety, minimal side effects on platelets, low plasma hemoglobin, minimal gas and particulate microemboli, and fewer visual-motor sequelae, as measured by the Bender-Gestalt test, noted in patients after 2.5 ± 1.5 hour perfusions with the Landé-Edwards membrane oxygenator compared with similar operations using the bubble oxygenator (10–12).

This communication describes the oxygen transfer observed during the recirculation circuit in our first 23 heart operations and during the presently preferred simple circuit of gravity flow in the next 87 heart operations.

*Recirculation:* The oxygen saturation and transfer data obtained during recirculation with 2 to 3 $M^2$ Landé-Edwards membrane oxygenators are shown in Figure 2. Venous inlet oxygen saturation levels ranged from 42 to 98%, arterial oxygen levels ranged from 88 to 100% at mean temperatures of 31°C (range of 30–33°). The mean hematocrit was 32% (range, 25 to 37). The mean body weight was 72 Kg (range, 60 to 103). Pump time for patients averaged 2.5 hours (range, 1 to 6.5). The mean oxygen transfer was 30 ml $O_2/M^2$ membrane/min (range 14 to 40). Maximum arterial oxygen saturations of 100% were observed during arterial extracorporeal blood flow rates between 2.75 and 4.25 L/min.

*Gravity Flow:* The oxygen saturation and transfer data obtained during gravity flow are shown in Figure 3. Two 3 $M^2$ Landé-Edwards membrane oxygena-

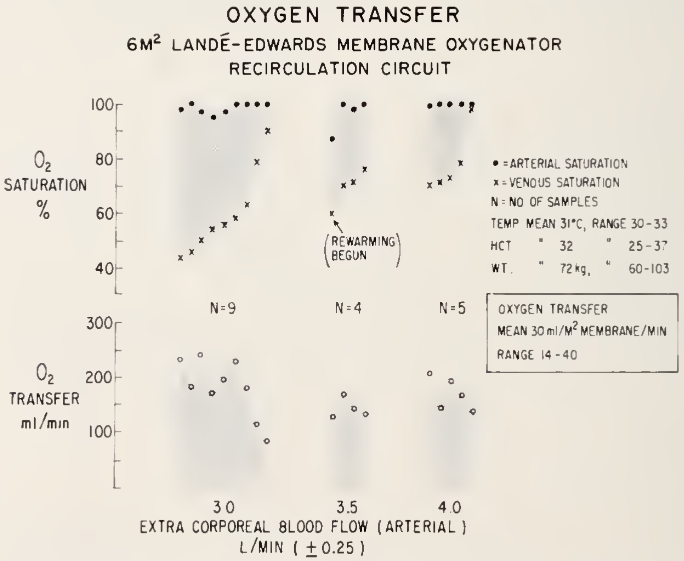

FIG. 2. Oxygen transfer with 6 $M^2$ Landé-Edwards membrane oxygenators in recirculation circuit. The mean oxygen transfer was 30 ml/$M^2$ of membrane/min (range 14 to 40); $O_2$ transfers were similar at arterial extracorporeal rates of 2.75 to 4.25 L/min. at mean hypothermic temperatures of 31°C, range 30 to 33°C. (This complex recirculation method was found to be unnecessary.)

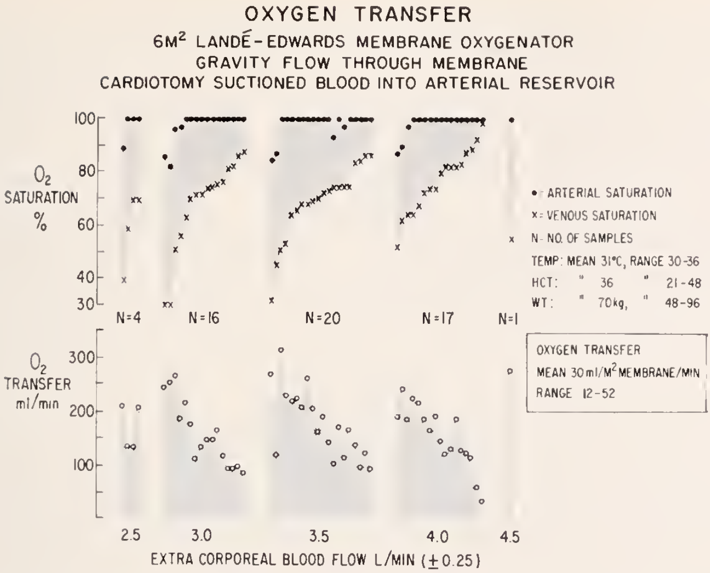

FIG. 3. Oxygen transfer with 6 $M^2$ Landé-Edwards membrane oxygenators with gravity flow of venous blood through membrane, and cardiotomy suctioned blood directly entering the arterial reservoir. Note that the mean oxygen transfer rate is 30 ml/$M^2$ of membrane/min (range 12 to 52) with similar mean temperature of 31°C and mean temperature of 31°C and mean body weight of 70 Kg as in the more complex recirculation circuit. The circuit presently recommended is seen in Fig. 1, where the cardiotomy suctioned blood enters the venous line. This allows the excess cardiotomy suctioned blood as found in certain valve and congenital heart operations to be oxygenated before entering the arterial line. In order to test the capacity of the oxygenators, several patients were gradually warmed while on total perfusion. Maximum $O_2$ transfer capacity of 35 to 52 ml/$M^2$ of membrane/min was seen with venous inlet $O_2$ saturations below 45%.

tors were connected to a circuit allowing venous blood to drain by gravity into the membrane units and cardiotomy suctioned blood to enter the arterial reservoir. (In our present method, used since March 1972, the cardiotomy suctioned blood enters before the membrane rather than after the membrane into the arterial reservoir.) Venous inlet oxygen saturation levels ranged from 30 to 98% and arterial oxygen levels from 80 to 100% at mean rectal temperatures of 31°C (range 30–36). The mean hematocrit was 36% (range 21–48). The mean body weight was 70 Kg (range 48–96). While using the gravity circuit, many of the patients had simple, short, operations. The pump times ranged from 0.5 to 4 hours, averaging 1.75 hours. Oxygen transfer rates were similar to those obtained when the more complicated recirculation circuit was employed. The mean oxygen transfer was 30 ml/$M^2$/membrane/min (range 12 to 52). Maximum arterial oxygen saturations of 100% were observed at arterial extracorporeal rates of 2.25 to 4.75 L/min. The lower oxygen transfer rates (below 100 ml $O_2$/min) occurred during hypothermia.

In order to test the oxygen transfer capacity of these membranes, we gradually used less and less hypothermia toward the end of several operations. This explains the low venous inlet saturations in a few patients. The arterial outlet saturation was 40 to 50% higher than venous inlet saturation when the inlet saturation was less than 60%. The capacity for oxygen transfer ranged from 30 to 52 ml $O_2$/min/M$^2$ membrane depending on the degree of inlet desaturation. During mild hypothermia of about 33°C, the oxygen transfer in 16 patients averaged 30 ml $O_2$/M$^2$ membrane/min. ± 15 (1SD).

## Discussion

Since March 1972 we have added the cardiotomy suctioned blood to the venous inlet rather than the arterial reservoir. This prevents arterial desaturation when large amounts of unsaturated blood are suctioned in certain valve or congenital heart operations. Similar oxygen transfer rates were measured in each gravity flow system. This presently used circuit of gravity flow, and venous inlet of cardiotomy suctioned blood, as shown in Figure 1, is similar to the simple circuit used with the bubble oxygenator.

Gas exchange was influenced by the weight, temperature, and amount of skeletal muscular activity of the patient. Because oxygen and carbon dioxide exchange across the membrane is limited, continued pulmonary ventilation and maintenance of cardiac contractions during cooling and rewarming appears advisable in larger patients unless more membrane is added. The additional time needed for partial perfusion during rewarming is considered to be more desirable than adding an additional membrane unit to provide the extra gas exchange needed for total perfusion during normothermia. One 3 M$^2$ unit per 40 Kg body weight provided adequate gas exchange. Two patients, 96 and 103 Kg, had satisfactory gas exchange with only two 3 M$^2$ units during an interval of ten minutes while the tubing of the third membrane was clamped. The arterial oxygen tension was maintained at 93 mmHg for those ten minutes at 30°C.

The recirculation method used in the first 23 patients was found to be unnecessary. Adequate oxygen and carbon dioxide exchange was obtained in the next 77 patients using the simple gravity flow methods.

## Summary

One hundred patients, 7 to 103 Kg, received total cardiopulmonary support with the disposable Landé-Edwards membrane oxygenator during heart surgery. Initially the membrane units were used only during complex aorto-coronary artery-vein graft operations, reoperations, or when low cardiac output dictated cardiopulmonary support prior to thoracotomy. (Pump time, 1 to 6.5 hours.) Later the units were used for routine open-heart surgery. Two 3 M$^2$ membrane oxygenators were linked in parallel to a standard roller pump apparatus. (Three 3 M$^2$ membranes for patients 80 to 103 Kg).

A simple circuit allowing venous blood to drain by gravity through the membranes into an arterial reservoir is preferred now. One roller pump returns the blood to the patient. Blood suctioned from the pericardial cavity and left ventricle enters the venous line via a cardiotomy reservoir and a 40-micron microfilter.

Oxygen transfer rates with 6 M$^2$ membrane oxygenators were a mean of 30

ml/$M^2$ membrane/min (range, 12 to 52). When venous inlet oxygen saturation was above 45% the arterial outlet oxygen saturation was 95 to 100% at flow rates between 2.25 and 4.75 L/min.

With moderate hypothermia of 30°C, oxygen metabolic needs were easily provided for by 6 $M^2$ membranes. With mild hypothermia of above 33°C, the transfer measured up to 300 ml $O_2$/min in larger patients. The capacity of the 6 $M^2$ Landé-Edwards membrane oxygenators was 30 to 52 ml/$M^2$ membrane/min depending on the inlet desaturation. For total perfusion normothermia in the larger patients one needs to add another membrane unit or preferably continue pulmonary ventilation and the heart beat during cooling and rewarming between 33° and 37°C.

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# Rationale and Early Experience in Assisted Circulation

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The successful development of extracorporeal circulation for use in open heart surgery led to the concept of using the same type of technique for the treatment of a variety of abnormal circulatory states. During the past fifteen years the progress in the field of assisted circulation has been painfully slow, as compared with the success of open heart surgery, and in many aspects the problems remain unsolved. The inherent complexity of the methods of circulatory assistance and the lack of definition of the specific indications for the different available methods are perhaps the reasons for the lack of progress. The purpose of this paper is to review the early experience and rationale for the use of assisted circulation in acute circulatory failure of cardiac origin. Since failure of the right heart is intimately related to pulmonary disease, which is discussed in another section of this symposium, we will confine this review to the problem of acute left heart failure.

## *Rationale of Assisted Circulation*

"Whenever the systemic blood cannot find a ready exit from the left ventricle, and when, at the same moment, there is no diminution in the supply of venous blood, an accumulation or congestion of blood must take place within the cavities of the heart" (1). In very simple terms, the above concept of acute heart failure, accepted for about 100 years, has led to two approaches to aiding the failing heart. In the first approach, blood is diverted from the left ventricle, using some variety of extracorporeal circulation technique. The method has been termed "flow assist" and its physiologic effect is to reduce the preload of the left ventricle. The second approach is aimed at facilitating the emptying of the ventricle by reducing the afterload. A pump synchronized with cardiac action, removes blood from the aorta during systole, decreasing the systolic pressure. The blood is returned during diastole, increasing the perfusion pressure during this part of the cardiac cycle. This modality of assist is generally known as "counterpulsation," or "synchronous pressure assist."

The physiologic rationale for the flow or bypass type of assist is deceptively simple. It has been suggested that any of the bypass techniques which reduce the flow load of the heart and the dimensions of the cardiac chambers will increase myocardial contractility by restoring a better length-tension relationship to the "overstretched" myocardial fibers (2). However, evaluation of the physiologic effects of the bypass type of assist in terms of the work of the heart and myocardial oxygen consumption has produced controversial results. Sarnoff et al (3), in a widely quoted work, indicated that the left ventricular work and myocardial oxygen consumption were mainly determined by the pressure load rather than the flow load of the heart. They further described the tension time

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index (TTI), a hemodynamic parameter related to the area under the systolic pressure curve, which showed an excellent correlation with changes in myocardial oxygen consumption. The TTI has been extensively used for the evaluation of different types of flow assist. Furthermore, the work of Salisbury et al (4, 5) suggested that almost the entire cardiac output must be bypassed from the heart before a noticeable decrease in oxygen consumption is observed. However, Dennis et al (6) have challenged the correlation between TTI and oxygen consumption, especially when the dimensions of the left ventricle change. In experiments of left atrial to femoral bypass, they demonstrated a decrease in oxygen consumption as the bypass flow increased, although the TTI did not decrease until practically the entire flow was diverted from the heart. Despite the discrepancies in results of the physiologic measurements, it is the common experience that in man, after open heart surgery, considerable hemodynamic improvement is obtained with even a modest amount of partial bypass.

The rationale for the use of counterpulsation as a method of temporary support is, on the other hand, relatively well accepted. Reduction of the systolic pressure decreases the left ventricular afterload and myocardial oxygen consumption (7). Elevation of the diastolic pressure provides additional beneficial effects to the failing heart. In the first place, there is an increase in coronary flow due to the increased coronary perfusion pressure during diastole (7). In some circumstances, elevation of the coronary flow helps in reversing cardiac failure (8, 9) and actually produces an increase in myocardial oxygen consumption by providing oxygen to areas of the myocardium previously under-perfused (10). There is, in addition, substantial evidence indicating that counterpulsation increases the blood supply to the marginal area of infarction (11, 12) and reduces the size of the infarcted area, at least in the experimental animal (13). Early work by Jacobey et al (14) indicates that increasing the diastolic pressure by counterpulsation appears to produce prolonged changes in the coronary vasculature by increasing coronary collaterals.

### *Clinical and Experimental Applications of Flow Assist*

The various methods which have been proposed to assist the circulation are listed in Table I. The initial approach to circulatory assist was partial venoarterial bypass with and without an oxygenator. The procedure was typically performed by withdrawing blood from the femoral or jugular vein into an extracorporeal circuit. The blood was then oxygenated and returned, by a pump, to the arterial system through a femoral or axillary artery.

The early experimental (15) and clinical (16) trials of Newman et al yielded occasional survivals in cases of right heart failure. Prolonged partial bypass with oxygenation resulted in undefined blood alterations related to the oxygenator (17) which limited the safe period of perfusion. However, even short periods of partial bypass have been shown by Kennedy (18) to be of value in the treatment of left heart failure. Partial bypass without oxygenation was investigated by Connolly and associates (19) in animals with acute right heart failure.

TABLE I

*Methods of Assisted Circulation*

- Flow Assist
  - Total cardiopulmonary bypass
  - Venoarterial bypass
  - Venoarterial bypass with oxygenator
  - Venovenous bypass with oxygenator
  - Left atrium to femoral bypass
  - Left ventricle to femoral bypass
  - Venopulmonary artery bypass
- Synchronous Pressure Assist
  - Counterpulsation
  - Intraaortic balloon counterpulsation
  - External synchronous pressure assist
  - Synchronous respiratory assist
  - Pulmonary artery counterpulsation
- Hybrid Systems
  - Synchronous venoarterial bypass
  - Synchronous left atrium to femoral artery bypass
  - Implantable cardiac compression cup
  - Inertial cardiac assistance

They demonstrated obvious relief of right ventricular overload in their preparation; but shunting of deoxygenated blood into the arterial tree has been a cause of concern to other investigators (20).

Direct cannulation of the left cardiac chambers, as in left atrium to femoral artery bypass, eliminated the need for an oxygenator in the presence of satisfactory respiratory function. In addition, it provided a more direct diversion of the volume load of the left heart. The technique developed by Dennis and co-workers (21–23) involved a transseptal cannulation of the left atrium via the internal jugular vein. The method has enjoyed only a limited clinical trial. Open chest direct cannulation of the left atrium following cardiac surgery was used by Spencer et al (24) in three patients. In general, all patients improved during left atrium to femoral artery bypass, but they died following termination of the procedure.

A new promising technique of left ventricular bypass has been proposed by Zwart et al (25). Blood is removed from the left ventricle with a flexible cannula inserted via the axillary artery. The blood is pumped back into the femoral artery in a pulsatile or nonpulsatile fashion. The technique is capable of achieving total bypass and appears to be an excellent method of assist if the pulmonary function is adequate, but clinical experience is as yet scanty (26).

Venopulmonary bypass was proposed as a method of circulatory assist in pure right heart failure (27, 28). The method requires a thoracotomy and offers no advantages over venoarterial bypass techniques. Venovenous bypass, although effective in providing oxygenation, is not a method of circulatory assistance.

In summary, closed-chest left ventricular bypass is perhaps the most promising shunt method of assisting the failing heart. However, partial venoarterial bypass, using improved blood-compatible surfaces and the new generation of membrane oxygenators, may prove to be the method of choice. Peirce (29) and others (30) have shown that prolonged extracorporeal circulation is possible in the experimental animal using a membrane oxygenator. Recent advances in control of anticoagulation therapy offer an added safety factor which should make possible a more definitive evaluation of the use of prolonged venoarterial bypass in the treatment of cardiogenic shock.

### *Synchronous Pressure Assist*

Counterpulsation offered a radically different therapeutic approach to the patient in cardiogenic shock. The technique involved bilateral femoral artery cannulation, and a single-ended pump, synchronized with cardiac action. Proper phasing of the pressure changes at the root of the aorta is essential. A very extensive and generally favorable experimental evaluation provided high expectations for this method of assist.

The initial clinical evaluation in a small group of patients resulted in occasional long-term survivors. The clinical problems related to the cannulation of severely arteriosclerotic femoral vessels limited the stroke volume of the counterpulsation pump and the effectiveness of counterpulsation. Prolonged pumping was attended by blood trauma (31) and the requirement for heparinization occasioned clotting problems similar to those encountered in partial bypass.

A partial solution to the above problems has been to perform counterpulsation by means of an intraaortic balloon. The balloon, introduced to the level of the descending aorta via a femoral artery, is inflated and deflated in synchrony with the heart, changing phasically the capacity of the aorta and thus producing counterpulsation effects.

The balloon counterpulsation, attempted originally by Clauss (33) and Moulopoulos (34), is today an accepted method of treatment of the patient in cardiogenic shock. Recent extensive clinical trials (35, 36) seem to indicate that perhaps one-third of the cardiogenic shock cases can be salvaged if the method is applied before the patient reaches a preterminal state. Further improvements in the design of the balloon system appear to provide increased safety and enhancement of hemodynamic effects (37). Prolonged assist is possible with minimal trauma or discomfort for the patients.

Even less traumatic, and completely noninvasive, is the method of synchronous pressure assist. In this technique, the counterpulsation effect is produced by a retrograde flow of blood squeezed from the lower extremities by synchronous external compression. The experimental basis of the method has been extensively investigated (38, 39). Detailed hemodynamic effects in patients undergoing cardiac cathetherization have documented in man the same hemodynamic effects obtained in the experimental animal (40). Initial clinical evaluation (41, 42) appears promising. The main advantage of the method is its noninvasive nature which permits earlier and easier application in the cardiogenic shock patient.

Counterpulsation of the pulmonary artery can be achieved by respiratory pressure-volume variation (43) or by direct pulmonary artery cannulation (44). Both are interesting experimental techniques with essentially no current clinical applications.

In summary, the pressure types of assist are today the most accepted techniques for assisting the circulation in cardiogenic shock. Early application is possible and less trauma is produced with external counterpulsation. However, balloon assist has been more extensively tried to date and can be combined with extracorporeal circulation for the operative treatment of certain cardiac shock states.

### *Hybrid System*

The combination of shunt and pressure assist would theoretically add the advantages of the two approaches and increase the effectiveness of the procedure. In synchronous venoarterial bypass, blood is returned to the arterial tree during diastole. Synchronous venoarterial bypass with counterpulsation requires more complicated equipment. The procedure is performed by returning the bypass flow into the arterial tree by a counterpulsation pump. To permit adequate withdrawal of blood from the aorta during cardiac systole, a positive acting valve must stop the inflow of bypass blood into the counterpulsating pump during this time (31, 45). In this method, the increase in diastolic pressure produced by the counterpulsation is enhanced because of the added stroke volume of the bypass flow. In addition, the assist can be carried out in the absence of effective cardiac action which, of course, is necessary for any pure pressure assist. The method requires multiple cannulations and complicated equipment. The main objection, however, has been the trauma produced by the extracorporeal devices. New developments in oxygenators and extracorporeal technology offer great incentive for a new evaluation of this method of assist.

Synchronous left atrium to femoral artery bypass was performed experimentally by Brown et al (45) by returning the blood during diastole in a pulsatile fashion. The pulsatile bypass did not significantly improve the hemodynamic effects produced by nonsynchronous, nonpulsatile bypass. In a similar way, left ventricular to femoral artery bypass has been achieved with a pump in which the return could be pulsed in synchrony with cardiac action.

Two additional methods of assisting the circulation are listed in Table I. Inertial cardiac assistance (47) is performed by accelerating the body in a longitudinal direction, thus accelerating the left ventricular and ascending aortic blood toward the head during systole. The method has had limited experimental evaluation. Mechanical direct ventricular assist (MVA) by means of a plastic cup applied directly to the heart has been extensively evaluated experimentally. The method has been shown to provide an effective cardiac massage for prolonged periods of time (48). Combination of MVA with balloon pumping has been shown not to increase the effectiveness of MVA alone (49).

## Comments

Recent clinical studies (35, 36, 42) have shown that possibly one-third of patients in shock due to myocardial infarction can be salvaged by the early application of counterpulsation. This represents only a minor improvement over the results of current medical therapy. In the remaining patients, presumably those with a severely damaged myocardium (50), temporary support is not sufficient to reverse the process of progressive deterioration and death. Until a satisfactory means of heart replacement becomes available, emergency revascularization, as advocated by Austen et al (36), would appear to be a promising approach in some selected patients. Although a reliable predictive index of the ultimate course of cardiogenic shock is not available, the pattern of response to assisted circulation suggests the feasibility of the following management. The patient in cardiogenic shock who fails to respond to medical treatment should be promptly supported with counterpulsation in the form of balloon pumping or synchronous external assist. If a sustained improvement is not obtained during the first few hours, partial venoarterial bypass with a membrane oxygenator, combined with counterpulsation, would provide the necessary life support system to permit the indicated diagnostic procedures and corrective surgery. In addition, all patients who are alive after a short period of temporary support should be evaluated for possible myocardial revascularization.

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# Membrane Oxygenator Assist in Cardiogenic Shock

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The need to provide mechanical assistance for patients suffering from cardiogenic shock has challenged a number of those engaged in membrane oxygenator research. The earlier enthusiasm for balloon pumping and other means of counterpulsation for cardiac support contributed to the delay in recognizing the efficacy of venoarterial support. However, with the inability of pure counterpulsatory systems to provide substantial support (greater than 20 percent) increase in total arterial flow, membrane oxygenator based systems may hold the key to future effective treatment of cardiogenic, as well as pulmonary derived shock.

## *The Logic of Venoarterial Assist*

The logic of venoarterial support for cardiogenic shock is based on its ability to double or quadruple delivery of oxygenated blood to the body with only peripheral intervention. Left heart bypass can be performed without an oxygenator but necessitates cannulation of the left side of the heart. Besides supporting kidney, liver, and brain functions, and decreasing lactate formation in the musculature, there is also augmented blood flow to the coronary arteries because of the increased mean pressure. Even arteries partially occluded by plaques or thrombi may be expected to function more effectively. With this help, even the ailing heart can contribute more to total output. The downward spiral of the deprived heart, where decreased output both to the body and the coronary circulation leads to progressive weakening of the heart, may then possibly be interrupted.

Venoarterial assist reduces the preload on the heart by removing a substantial portion of the blood which is returning to the failing heart and threatening to overwhelm its capabilities. By reducing the volume of each chamber one prevents overstretching the failing muscle. Additionally, dangerous back pressures on the lung, which might cause pulmonary edema, may be obviated.

An argument against the efficacy of venoarterial bypass in treating cardiogenic shock relates to its theoretical failure to reduce pressure work. Venoarterial support may be so effective in restoring normal aortoarterial hemodynamics that the weakened heart has an even more difficult time ejecting blood in the face of the increased afterload and an increased left ventricular end diastolic pressure (LVEDP) may occur. This has occasionally been seen in the most severely affected hearts, although most others have been able to superimpose good pulse pressures on the pressures produced by nonpulsatile venoarterial pumping. Fortunately, workers with counterpulsation have provided us with a number of devices which can be utilized to reduce this disadvantage should it arise. By combining an intraaortic balloon pump with venoarterial

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bypass and programming the balloon to collapse during systole, cardiac ejection is facilitated and excessive overload avoided. If a pulsatile pump is utilized to power the venoarterial circuit and this is properly triggered to inject mainly during diastole, then competition with the heart is further minimized (Figs. 1 and 2).

Whether such a logical system will, in fact, be effective in reducing morbidity and mortality will depend on the overall medical-surgical setting which surrounds it. Patient selection is the most important factor that will determine eventual success. Cardiogenic shock must be diagnosed early—ideally while it is still impending—and vigorous medical therapy with diuretics, oxygen, and suitable cardiotonic and antiarrhythmic drugs carried out. If dramatic response is not obtained, mechanical assist should be instituted immediately to prevent a downward spiral. Definitive diagnostic tests, including cineangiography and cardiography, can be carried out with the patient on assist. Informed decisions about the nature of combined medical-mechanical-surgical care can thus be made and carried out in a situation which demands rapid action. Patient selection to date has been of candidates whose expected mortality was essentially 100 percent and which one hoped to reduce to perhaps 90 percent. The yield of long-term survivers has understandably been poor. Not until we recognize and act on a group of patients with a better prognosis, and earlier, will a substantial number of lives be saved.

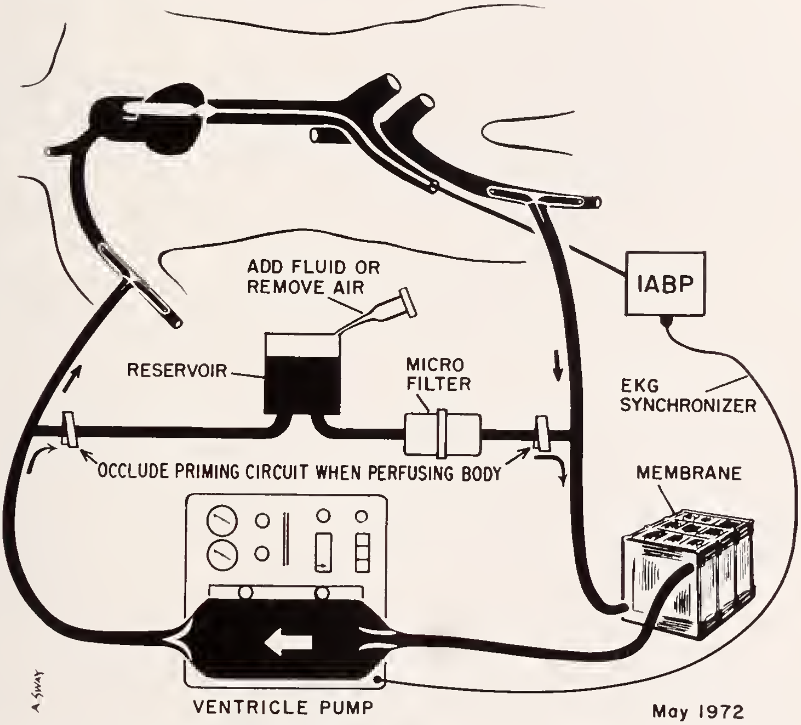

Fig. 1. Simple circuit for cardiopulmonary assist includes membrane oxygenators, ventricle pump, and optional intraaortic balloon pump.

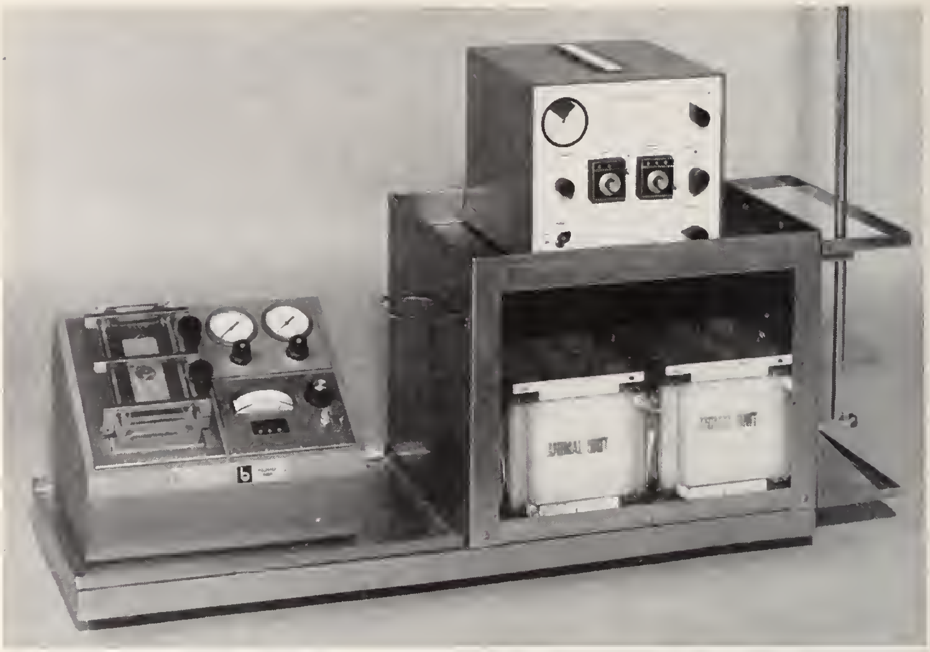

FIG. 2. Components of synchronous assist system.

### *Experience with Venoarterial Assist*

Every experienced cardiac surgeon has had patients who failed to "come off pump" after operations, who responded to partial assist bypass with relatively normal hemodynamics, and who could eventually be taken off assist following the passage of time and adjustment of a variety of factors. The efficacy of such assist in the operating room being taken for granted, it only required the advent of convenient membrane oxygenators to make prolonged bypass for cardiogenic shock a realistic area for investigation. Even before membrane oxygenators were generally available, attempts at prolonged bypass were made with improvement in hemodynamic factors and urinary output. Occasionally a patient survived.

Venoarterial perfusion of some 20 patients, the etiology of whose cardiogenic shock ranged from myocardial infarction to heart transplant rejection, produced a uniformly good hemodynamic response while on partial venoarterial bypass except in instances where shock had been of long-standing duration. In these, it was impossible to maintain arterial pressure no matter what flow was supplied. A number of patients, taken off bypass after being hemodynamically stable for days, relapsed into shock in a few hours. One patient in shock for 18 hours was improved sufficiently during 12 hours of bypass to be returned to the general medical floor, but a new embolic episode resulted in sudden death two weeks later. The occurrence of an elevated LVEDP in the most severely affected patients and the complexity of conventional operating room pump consoles, which require trained perfusionists and surgeons in attendance at all times, have prevented early acceptance of venoarterial bypass in the treatment of cardiogenic shock. Perhaps simpler techniques and the incorporation of means for dealing with an elevated LVEDP will remedy this.

The realization that gravity is ample to drive blood through membrane oxygenators connected in parallel has done much to simplify our technique. Single-pump circuits based on this principle are in daily use for longer cardiac surgical procedures. Some further simplification will be possible through use of combined heat exchanger-reservoirs. The membrane oxygenator, a heat exchanger-reservoir, and a single pump will comprise a circuit for perfusion, and all blood-contacting parts will be sterile and disposable.

For prolonged assist procedures the classic roller pumps have certain disadvantages. They are almost always operated in an occlusive mode and are driven by powerful motors which must be capable not only of overcoming resistance in the arterial circuit but also of serially compressing thick-walled plastic tubing. Consequently, they are inherently insensitive to decreases or increases in the amount of blood to be pumped and to restrictions to inflow and outflow. Unless supplemented by complex saftey devices (which are inherently untrustworthy) or constant vigilance and instantaneous reaction of perfusionists, introduction of air into the patient, or disruption of lines represent constant dangers. Therefore, we are most interested in the development of convenient ventricle pumps which utilize gravity filling of compliant ventricles whose pistons are powered by safe levels of gas pressure. One such pump which we are testing does not require even a reservoir in a basic support circuit consisting only of membrane oxygenators in parallel, a heat exchanger, and a ventricle pump. Most important, these components are small enough to be placed under the patient's bed, and safe enough to function with less supervision than is required by a respirator.

### *Routine Cardiac Surgery with Membrane Oxygenators*

Controversy greets the proposition that membrane oxygenators should replace the usual disposable bubble types for routine operations. Why, say many satisfied users of bubble oxygenators, should one change to a system employing membrane oxygenators which are more costly and have marginal gas transfer capability at normothermia, thereby delaying rewarming until intracardiac manipulations are completed? Why should one shift away from a system which has proven itself in tens of thousands of perfusions? It appears that change to the membrane is more readily acceptable where particularly long procedures are planned, especially in coronary artery surgery which can be accomplished with minimal hemo-destructive cardiotomy suction. Membrane oxygenators are more physiologic than direct blood-gas interface types, having the capability for perfusions lasting weeks as opposed to hours. But, what advantage is there in using the membrane oxygenator for valve replacement or pediatric correction when available comparative studies have not shown a clear-cut superiority? Obviously a definitive case for routine use cannot be made until factors such as cardiotomy suction systems are modified and large prospective comparative series of patients have been completed.

On the other hand some studies presented so far which have yielded indeterminate comparisons between short bubble and membrane runs have been, at best, rather crude yardsticks since small numbers of procedures have been involved and cardiotomy suction causing hemolysis has clouded the results.

The capacity of membrane oxygenation to be carried on for weeks as opposed to hours which has been proven in nonsurgical laboratory and clinical situations is significant. Even the most routine cardiac surgical procedure can unpredictably require extended perfusion due to unforseen circumstances. At that point, a capability for long-term bypass could make the difference between life and death, a difference which is not sufficiently reflected in all-inclusive statistical groups.

A variety of laboratory studies, indicating decreased blood damage with use of the membrane even for short periods, and the admittedly not completely benign nature of conventional bypass, demonstrated by many indications that "closed" operations are safer than "open" operations, leads one to conclude that short-term membrane bypass should be developed and studied.

Two technical improvements might make the advantages of the membrane oxygenator for short-term bypass less obscure. The surgeons should turn the suction down, and turn it off when it is not actually needed, by means of foot operated on–off and speed control switches. A bubble oxygenator, fitted with an access port which bypasses its oxygenating portion, can beneficially be used as a heat exchanger-reservoir during hypothermia. The oxygenating portion of the bubble oxygenator will be available to supplement gas exchange during rewarming if defibrillation must be delayed. Fewer membrane oxygenator units would suffice for this mode of operation.

Membrane oxygenators have certain unique characteristics which give them a number of unusual capabilities. Excellent control of patient's volume can be maintained with the extremely constant volume membrane oxygenators as opposed to variable volume bubble and screen types. Postoperative complications in the trachea and lung currently loom as important deterrents to early complete correction of many defects. Volume control, along with presumed decreased damage to end organs, such as lungs and kidneys, by minimally denatured blood, makes intubationless open heart surgery on tiny infants, with or without hypothermic arrest, a tantalizing possibility.

These arguments are advanced in order to counteract the equivocal impression which may have been left by the few small comparative series which have been reported to date, which have not yielded definitive results. Further development and more widespread use are needed to explore the many ways in which the advantages of membrane oxygenators can be utilized every day in the operating room, as well as for occasional nonoperative support.

# Prolonged Respiratory Support in Infants with the Kolobow Spiral Coil Respirator

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Patients with temporary overwhelming respiratory failure often need prolonged respiratory support. Mechanical ventilation is frequently inadequate and high oxygen tensions of the ventilating gas may damage the lungs. Another approach to partial respiratory support may be extracorporeal membrane respiration.

Over the past few years, our in vitro (1) and animal (2, 3) evaluation of the spiral coil membrane oxygenator designed by Kolobow (4, 5) has demonstrated: 1) That it provides excellent oxygenation and $CO_2$ removal; 2) That it is relatively innocuous to the subject; and 3) That it is capable of providing long-term respiratory support. With this background, we decided to offer such support to patients who were dying from respiratory insufficiency when ordinary techniques of support were used. Since the prototype 0.4 $M^2$ membrane respirator appeared to be best suited for respiratory support in small infants, suitable patients for trial of the membrane respirator were sought among neonates with the respiratory distress syndrome due to hyaline membrane disease.

This paper summarizes our initial experiences with extracorporeal respiratory support with membrane respiration for ten, three, and two days in three newborn infants. The evolution of our current approach to techniques of extracorporeal respiratory support, and to criteria for patient selection are also presented.

## General Management

Prior to the decision to use extracorporeal membrane respirator support, an umbilical artery catheter was placed in the descending aorta for sequential blood gas sampling, and constant blood pressure monitoring. Pulse and respirations were monitored electronically. Infants were selected by the senior neonatologist responsible for their care and not by the respirator support team.

Once the decision for support was made, a right internal jugular vein cutdown was performed. A Silastic® catheter (1.98 mm I.D.; 3.175 mm O.D.) was positioned in the superior vena cava for withdrawal of blood to the oxygenator. Another cutdown was performed on the abdominal wall a short distance cephalad to the umbilicus, and the umbilical vein was cannulated with a larger Silastic catheter (3.35 mm I.D.; 4.65 mm O.D.) for return of oxygenated blood. Both cannulas had sidearms with 3-way stopcocks for blood sampling, and were filled with heparinized saline and clamped until the bypass was connected. We

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selected veno-venous bypass in these neonates since vessels of a caliber sufficient for cannulation by wide bore catheters were available through a relatively minor and non-traumatic procedure (Fig. 1).

While the venous cannulations were performed, the sterilized membrane oxygenator bypass system was assembled. The initial prime was of lactated Ringer's solution, during which all air was expelled from the system and good pumping flow established. The crystalloid was then replaced with fresh, heparinized blood. The total priming volume was about 200 ml, 50 ml for the 0.4 $M^2$ oxygenator, and approximately 150 ml for the tubing. When completely set up, the system was connected to the venous cannulas in the infant. From the superior vena cava withdrawal cannula, venous blood fell by gravity to a Silastic reservoir from which it was pumped by a semi-occlusive pump to the membrane oxygenator. The oxygenated blood from the oxygenator flowed through a bubble trap before returning to the infant via the umbilical vein cannula.

In our experience, the spiral coil membrane respirator is most efficiently ventilated by a mixture of oxygen and carbon dioxide. In our early cases, a Y-connector between a 100% $O_2$ source and a 95% $O_2$/5% $CO_2$ source allowed a calibrated blend of ventilatory gases. Generally, 4% to 5% $CO_2$ was required to maintain normocarbia and a normal arterial pH. The ventilating gas was delivered at 5 to 6 L/min into one cannula of the membrane oxygenator envelope, and pulled through the envelope by a vacuum of 200 mm Hg applied to the other end cannula.

The infants were treated in a specialized neonatal intensive care unit. Temperature was maintained by a radiant heat cot for the infant, and heat lamps to the extracorporeal setup as needed to counter radiant heat loss in the external circuit. Intravenous alimentation could be delivered into the sidearm of the umbilical vein return cannula. Antibiotics (cephalin and gentamycin) were routinely administered.

Bypass flow was adjusted to the adequacy of arterial oxygen saturation in the infant assessed by aortic blood gas sampling, blood pressure, urine output, and neurologic status. This, in turn, depended on the rate and depth of ventilation, and the adequacy of respiration across the diseased alveolar membrane in the presence of A-V shunting. A $Po_2$ of 50 mm Hg and a systolic B.P. of 50 mm Hg are normal for a newborn premature infant. Occasional blood gas samples were taken on both sides of the membrane oxygenator to determine the efficacy of oxygen transport across the membrane. The bypass flow could be reduced and ultimately discontinued as the infant's own ventilation and respiration recovered sufficiently to support him unaided.

Currently, heparinization is required to avoid clotting in the oxygenator system, and must be carefully controlled to prevent bleeding from overheparinization. Inasmuch as there are 4.5 units/ml of heparin in heparinized bank blood, the priming volume of blood alone constituted more than a complete heparinizing dose for a premature infant (e.g., 200 ml blood contains 900 units of heparin). Judicious protamine administration into the umbilical vein or

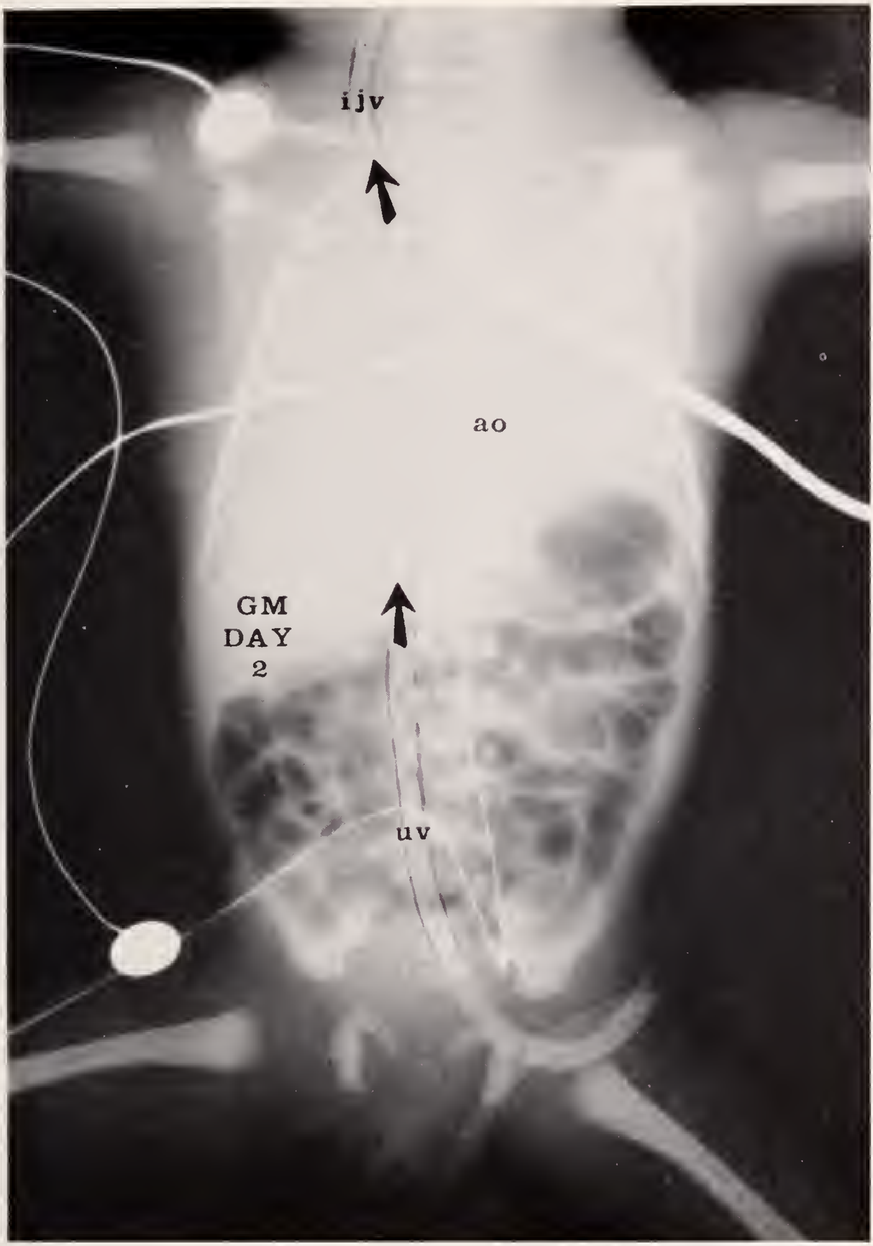

FIG. 1. Roentgenogram of Case 2 on the second day of extracorporeal membrane respirator support. The lungs are completely opaque. The cannulation positions are illustrated. Venous blood was delivered to the respirator via a cannula threaded through the internal jugular vein (ijv) into the superior vena cava; the oxygenated effluent blood was returned via a cannula stationed in the umbilical (uv.). A monitoring cannula has been passed via an umbilical artery into the aorta (ao).

aortic catheter was necessary. Thereafter, heparin was added to the reservoir as needed to maintain an appropriate clotting time.

Incremental blood replacement was necessary to counter oozing or bleeding from overheparinization, and the mild blood element breakdown from pumping and exposure to high oxygen tensions in the oxygenator. An average replacement of 3 ml/hour has been necessary in our early experience. Accumulation of fibrin and platelets on the oxygenator membrane may also have contributed to cellular breakdown. Routine replacement of the membrane oxygenator at 4 to 5 days seemed advisable.

## Clinical Material

Three infants with overwhelming respiratory distress have been treated with membrane respiration in our early application of this technique (6). All were premature, of 28, 30, and 32 weeks gestation, and 1000, 1250, and 1750 grams birth weight respectively. All had minimal chance for survival.

The first case was started on bypass at 12 hours of age, and almost immediately ceased spontaneous ventilations. Bypass flow was increased to 125 ml/min (approximately cardiac output), and maintained at this level of total bypass for six days. On the seventh day shallow spontaneous ventilations resumed, and the bypass flow was halved. Throughout the first week of bypass, the infant had good neurologic function. Eye movements and sucking were present, as well as voluntary and reflex movements of the limbs. In the 9th day, however, the baby abruptly became unresponsive, and stopped voluntary breathing. His blood pressure gradually deteriorated and urine output ceased. He died at the end of the 10th day. Postmortem examination showed subdural cerebellar and basal hematomas in the brain. The brain itself was extremely soft and necrotic. The spinal cord was also hemorrhagic. Generalized edema and jaundice were present, and the liver was congested. The first week of this extracorporeal bypass represents one of the most prolonged periods of total extracorporeal respiration yet reported in humans.

In the second case, partial respiratory support with the membrane respirator and venovenous bypass was started on the third day of life, as the probability of survival score dropped successively from 50%, to 33%, to 16%, the latter despite intensive bag breathing with 100% oxygen. Arterial pH was 7.08; $P_{CO_2}$ was 130 mm Hg; and $P_{O_2}$ was 25 mm Hg. Chest roentgenogram showed complete lung opacity (Fig. 1). The bypass flow was maintained at 60 to 70 ml/min (approximately half the estimated cardiac output) for the first 36 hours. The baby had shallow ventilations for the first 24 hours, which became stronger and deeper on the second day. A systolic blood pressure of 50 to 60 mm Hg was generally present. Urinary output, however, was minimal in the first 1½ days (32 ml), following which there was none. For the first 36 hours, the infant showed normal movements, opened his eyes, suckled, and cried. Twitching movements of the extremities started at about 36 hours and areflexia gradually supervened. Because of cessation of respirations, bypass flow was increased to 130 ml/min. There was no further urine produced from the 30th hour despite ethacrynic acid and mannitol administration. The baby died at 80 hours. Postmortem examination showed intracerebral hemorrhage, especially in the posterior part of the brain, and edema; there was moderate spinal cord hemorrhage. Acute tubular necrosis of the kidneys was present, as well as bilateral congenital renal artery stenosis.

In the third case, partial respiratory support was started at 14 hours of age. Initial bypass flow was 30 ml/min. By the 6th hour, a bypass flow of 130 ml/min had achieved blood gases in the normal range. After the first hour of bypass, endotracheal intubation, and a Bird ventilator were also used in an effort to encourage lung expansion and early return of spontaneous ventilations. For the first 18 hours, the infant had normal movements. After the 18th hour, the infant developed severe seizures and became completely unresponsive. Systolic blood pressure, which had been 50 to 60 mm Hg, gradually dwindled; there was a consumptive coagulopathy. Cardiac arrest and death occurred in the 40th hour. Postmortem examination showed extensive focal hemorrhages of the lungs, thymus and gastrointestinal tract. Fibrin thrombi were present in the intrahepatic portal radicles and sinusoids. Subdural and subarachnoid hemorrhages were present in the brain, particularly in the posterior fossa and midbrain.

## Evaluation

Excellent prolonged extracorporeal respiratory support was provided by this venovenous bypass and membrane respiratory system. Throughout the entire bypass period in all three cases, oxygen transport across the membrane oxygenator was complete, with 100% hemoglobin saturation and an average $Po_2$ over 300 mm Hg in the effluent blood (Table I). Oxygen transport, calculated for the standard membrane area of 1 $M^2$, was 38 to 45 ml/min/$M^2$ at bypass flows of 130 ml/min (Cases 1 and 3), and 25 ml/min/$M^2$ at 70 ml/min flow (Case 2). Actual oxygen delivery to the infants depended on the volume of bypass flow, and was 24, 13, and 20 ml/min respectively in the three cases. Since full

TABLE I

*Oxygen Transport and Delivery*

| To Oxygenator | | From Oxygenator | | Hematocrit (%) | Bypass Flow (ml/min) | $O_2$ Transport across Membrane (ml $O_2$/min/$M^2$) | $O_2$ Delivery to Patient (ml $O_2$/min) |
|---|---|---|---|---|---|---|---|
| $Po_2$ (mm Hg) | Sat'n (%) | $Po_2$ (mm Hg) | Sat'n (%) | | | | |
| | | Case 1 | | | | | |
| 45 (37–59) | 24 | 300 (108–495) | 100 | 40 | 130 | 45 | 24 |
| | | Case 2 | | | | | |
| 42 (31–59) | 21 | 410 (330–430) | 100 | 40 | 70 | 25 | 13 |
| | | Case 3 | | | | | |
| 48 (36–54) | 25 | 305 (146–430) | 100 | 36 | 130 | 38 | 20 |

Numbers given represent means.
Numbers in parentheses denote range.

saturation of the effluent blood was achieved, oxygen delivery to the patients was actually a function of the volume of bypass flow in these instances. Carbon dioxide removal was so efficient that respiratory alkalosis ensued. Since this may produce a "paradoxical" acidosis (1), 4 to 5% $CO_2$ was added to the ventilating gas for the unit to provide an arterial pH in the normal range. In fact, a mild respiratory acidosis (pH $\approx$ 7.3) and hypercarbia ($P_{CO_2} \approx$ 40 to 50 mm Hg) was selected at times by elevating the $CO_2$ concentration of the ventilating gas in order to provide a stimulus for spontaneous ventilation in the patients.

Spontaneous ventilations ceased in two infants shortly after bypass was started and were diminished in one. This did not occur in our animal experiments. The venovenous bypass technique mimics somewhat the fetal circulation inasmuch as oxygenated blood is returned via the umbilical vein. In premature infants, this may trigger a primitive reflex which stops ventilation.

Systemic heparinization is a requisite for the extracorporeal system and has provided some serious problems. Overheparinization can occur, particularly from the initial heparinized blood prime. Oozing from cutdown sites and frank GI hemorrhage may occur. This can be of particular consequence in the prematures with respiratory distress syndrome (RDS) who exhibit an increased tendency to intracerebral hemorrhage. Maintenance of a 30–40 minute clotting time and judicious protamine administration may ward off these sequelae.

Some destruction of blood elements does occur with extracorporeal bypass. A leukopenia ($\approx$ 2,500/$mm^3$) and thrombocytopenia ($\approx$ 50,000/$mm^3$) were present as well as abnormal erythrocytes on smear, and a gradual decline in hematocrit unaccountable by bleeding. There was a concomitant elevation in indirect reacting serum bilirubin ($\approx$ 10–12 mgm %). Such gradual damage was noted as well in our animal experiments, and may be due to trauma from the occlusive pump used in these cases or to toxic damage from the high oxygen saturation in the effluent from the oxygenator. Less traumatic pumps and provision for lower but adequate oxygen saturations are currently incorporated in our technique.

Efforts are continuing in our laboratories to improve the efficiency of the membrane respirators and enhance safety provisions. With the assistance of Sci-Med, Inc., who manufacture the Kolobow spiral coil membrane respirator, we are evaluating the use of positive pressure ventilation to the unit without added vacuum withdrawal. Maintaining the pressure in the blood compartment moderately higher than the air channel pressure provides excellent gas exchange and negligible possibility for air embolus.

It is frequently clinically necessary to use blood flows for these units significantly less than their full rated flow capacity. At slower flows (e.g. 25% or 50% of maximum value), there is a prolonged "residual time" of blood in the unit. At full ventilatory gas flows, a "hyperventilation" effect may be produced with resultant hypocarbia. Possible solutions involving appropriately reduced ventilatory gas flows consistent with full oxygenation, and/or the addition of $CO_2$ are being evaluated. At these flows, $P_{O_2}$ values far in excess of those required to obtain full hemoglobin saturation may be achieved as well. Exposure to these high $Po_2$ values (avg. 300 mm Hg in the cases cited) may be toxic to the red cells in the effluent blood until mixed with the main blood stream. Reduction of oxygen saturation in the ventilating gas consistent with full saturation is also being assessed. Suitable and accurate gas valving is essential.

Clinical indications for the use of extracorporeal respiratory support in neonates must be carefully elucidated. Our current approach is outlined in Table II, indicating several conditions we feel may be amenable to such therapy. It must be emphasized that such patients must be those who, in the judgement of the attending neonatologist, cannot be supported by individualized intensive respiratory care including positive pressure airway breathing and/or mechanical ventilation. Our indications for possible application of such therapy in babies with the Idiopathic Respiratory Distress Syndrome (IRDS) have been entirely reassessed since our original publication (6). At that time we utilized the probability of survival scores advanced by Swyer. With the advent of positive airway breathing, and, in particular, nasal positive-end-expiratory pressure breathing, we have paused until sufficient experience could be gained with these modalities. Some current statistics from the Newborn Intensive Care Unit of The Baltimore City Hospitals is presented in Table III. On this basis, an infant with RDS who cannot be managed by positive airway pressure breathing, and who has an arterial $Po_2$ less than 50 mm Hg on 100% $O_2$ inspired air would appear to be a suitable candidate.

Another group of neonates that may be suitably treated with extracorporeal oxygenation are those with the syndrome of "fetalization of the pulmonary

TABLE II

*Indications for Extracorporeal Respiratory Support in Neonates*

Infants with:
1) Idiopathic Respiratory Distress Syndrome
2) Hypoperfusion of Lungs
3) Congenital Heart Disease with Severe Cyanosis
4) Postero-Lateral Diaphragmatic Hernias with Hypoplastic Lungs
5) Massive Aspiration Syndrome
6) Severe Intrauterine Pneumonia

who cannot be supported by individualized intensive respiratory care including continuous Positive Airway Pressure and/or Mechanical Ventilation.

TABLE III

*Intensive Care Nursery Baltimore City Hospitals*
*(July 1971–June 1972)*

☐ 101 Infants with Respiratory Distress Syndrome

☐ Overall Mortality Rate (all weights)—20%

☐ Mortality Rate (>1000 gms)—9.5%

☐ Few Survivals <1500 gms on Ventilator

TABLE IV

*Indications for Extracorporeal Respiratory Support in Infants and Children*

☐ "Pump Lung"
☐ Drowning
☐ Smoke inhalation
☐ Overwhelming Pulmonary Edema
☐ Aspiration Pneumonitis
☐ Pulmonary Contusion
☐ Overwhelming Pneumonia (bacterial/viral)

arteries." These full-term babies have a low Apgar and severe hypoxemia with the rapid development of metabolic acidosis, even on ventilators and 100% oxygen. Half die, and demonstrate only a large patent ductus arteriosus at autopsy. Infants with severe congenital cyanotic heart disease (e.g., transposition of the great vessels), and babies with hypoplastic lungs associated with congenital postero-lateral diaphragmatic hernias may be suitable candidates as well.

Massive aspiration, and overwhelming pneumonias are other conditions which may require extracorporeal support both in neonates, and in infants. Other conditions found in infants and children with the potential for such support are listed in Table IV.

## Summary and Conclusions

Prolonged extracorporeal respiratory support with a membrane oxygenator provided excellent oxygenation and $CO_2$ removal and satisfactory support for two to ten days for three premature infants with fatal respiratory distress. Problems with heparinization and some blood element destruction were encountered. Further refinements in apparatus and technique are in progress. With suitable guidelines, prolonged clinical respiratory support with a membrane oxygenator appears to be feasible in carefully selected cases.

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# Respiratory Intensive Care: A Review with Possible Indications for Membrane Oxygenator Support

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> Ethical and practical problems limit knowledge of the disturbances of function in many of the acute emergencies of clinical practice, for investigation must not be allowed to hinder life-saving treatment. And lack of precise knowledge of what we are treating inevitably makes it more difficult to devise a better treatment.
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A dilemma that confronts the physician with a new, aggressive, and possibly complicating treatment for some life-threatening condition is that, in the initial stages of development, it will be applied only to those in extremis. It is not surprising, therefore, that when many or most of the patients die, the treatment falls into disrepute. If the new therapy is applied too lightly, or when unnecessary, the patient may now succumb to iatrogenic interference, rather than his disease, and again the treatment falls into disrepute. An incomplete or unavailable picture of the disease state itself, and frequently ignorance of the proper indications and complications of current remedies compound the problem with acute grave physiologic disturbances.

The use of the membrane oxygenator for the long-term support of the patient with severe acute cardiorespiratory failure has now saved some lives. The purpose of this article is to review some of the other modalities of treatment—their use, indications, and complications. With the knowledge of other modalities, it may be possible to ascertain that a patient has been given adequate treatment so that, if he does not improve, this new form of support should be employed. The sort of problem that is being considered is failure of a patient to maintain sufficient oxygen uptake to meet the needs of his metabolism. It is, therefore, proper to list the types of conditions that might adequately be ameliorated by bypass support. These must, at present, have a sufficiently brief phase of physiologic decompensation, so that if bypass is used, the patient will be well enough to manage on his own within about a week. The condition must either be self-limiting or treatable.

## *Reversible Cardiorespiratory Conditions*

(1) Infections: Acute bacterial pneumonias, viral pneumonias, and the rare protozoan Pneumocystis Carinii pneumonitis.

(2) Noninfectious: Chemical or inhalation pneumonitis, early (exudative phase) pulmonary oxygen toxicity and multiple small pulmonary emboli.

(3) Respiratory distress syndrome: Adult ("shock lung") or neonatal.

(4) Cardiac: Cardiac failure, particularly if originating from myocardial infarction or cardiac surgery, or compounded by hypoxemia.

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If the patient continues to fail when the cardiorespiratory resuscitologist* has emptied his cupboard of conventional fare, there is now a new remedy to be tried. What should be tried first?

### *Oxygen*

The best established remedy for hypoxemia is to increase the inspired oxygen tension, but the level should be kept as low as is compatible with an adequately oxygenated patient. The danger of breathing excessive oxygen concentrations was first discovered before the beginning of the last century (1–3). Biochemical changes with pure oxygen and cerebral symptoms with hyperbaric oxygen were described in 1878 (4). In 1889, pneumonic changes were described in the lungs of mice kept in one atmosphere (1 ATA) of oxygen (5).

Another possible complication of prolonged pure oxygen breathing is development of atelectasis in temporarily blocked portions of the lung. At least 15 percent of inert insoluble gas would seem to be desirable to help prevent this (6). Normal healthy man is fairly resistant to pulmonary oxygen toxicity, so it was not until the last decade that it was appreciated how often oxygen toxicity occurred clinically (7). Most data would suggest that pulmonary oxygen toxicity will not present a problem if the inspired oxygen tension is below 425 torr (about 60 percent of 1 ATA) (7, 8). Ciliary action and the clearance of mucus are diminished at even lower values. It may be that severely hypoxic patients are more resistant (9). The length of time needed before oxygen toxicity develops is uncertain. Roentgenographic changes attributable to oxygen toxicity can be demonstrated in three days (10). The exudative lesions that occur in the first four to five days are considered reversible while the later proliferative changes are probably not (11, 12).

The experience of the Mount Sinai Medical Center in New York with hyperbaric oxygen shows that very high oxygen tensions can be of value in acute respiratory failure, particularly if the condition is accompanied by myocardial hypoxia. The rationale (13) is to oxygenate the patient in the hyperbaric chamber, and try to restore pulmonary and cardiac function sufficiently to permit return to safe tensions of oxygen before pulmonary toxicity vitiates the improvement. Toxicity develops much faster at therapeutic hyperbaric levels, probably in 24 to 36 hours or less (7, 8). To date, 22 patients with severe acute respiratory insufficiency have been pressurized. Fourteen have improved (one after membrane oxygenator support) and returned to the normal intensive care units. Of these, five left the hospital well. Those who were unable to survive without hyperbaric oxygen generally had advanced pulmonary oxygen toxicity. The findings in the majority of these patients have been reviewed admirably elsewhere (10). Most of the experience in this series was gained before positive end expiratory pressure became a routine.

Another use of a large hyperbaric facility is that hypoxic patients can be kept alive safely while bypass support is being set up. This has been done twice at Mount Sinai.

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* Word unashamedly borrowed from Dr. Peter Safar.

### *Oxygen Consumption*

If the oxygen consumption can be reduced, the amount of cardiorespiratory failure that can be tolerated will be increased. The work of Hedley-Whyte and Winter (14) shows that morphine in large doses and muscle relaxants can reduce oxygen consumption to 25 percent below resting values. The reduction of fever, while shivering is controlled, is another obvious method, but actual hypothermia may not be as beneficial as might be expected. Pulmonary surfactant, for example, is much more readily exhausted at subnormal temperatures, particularly with the large tidal volumes used in patients with advanced lung pathology (15).

### *Pulmonary Function*

Getting the lungs into the best state possible to carry out gas exchange is a sorely neglected area of therapy outside centers which specialize in acute respiratory care. It is very hard work and requires many man hours per day. It is not too much for the physician to require at least a one-to-one ratio of trained staff to patient, as respiratory patients are the heaviest nursing load of all. Less help will lead to life-threatening compromise. Many centers have achieved this degree of support but it has been hard to convince most hospital and nursing administrations of the need. Intensive nursing care is expensive, but crucial to good rates of patient salvage.

A good airway is essential and the staff must be able to reestablish without delay one that has failed. At all times, there should be personnel on the spot capable of changing an endotracheal or tracheostomy tube. Endotracheal intubation, in skilled hands, can provide a much less traumatic airway than a tracheostomy, possibly for several weeks in a well-sedated patient. Expert endotracheal intubation provides a far lower mortality and morbidity (16), and it leaves the patient with a more effective cough to clear secretions when he is supporting his own ventilation. In inadequately staffed areas, earlier resort to tracheostomy is probably justifiable, as the less experienced may be caring for the patient.

Turning from side to side every two hours, clearance of secretions, chest physiotherapy, monitoring, and providing adequate humidification are all part of the time-consuming duties of the attendant staff. The intelligent use of the stethoscope to check aeration should be part of the nursing routine. Appropriate antibiotic therapy, with daily bacterial and fungal culture of secretions and blood should have high priority. With the use of antibiotics, it is often much harder to withhold inappropriate therapy than to use it.

Chest X-Rays should be routine. They provide early diagnosis of pneumothorax and collapsed lung, easily remediable but often missed complications.

The interrelationships of interstitial pulmonary edema, closing volume, functional residual capacity, central blood volume, pulmonary venous admixture and cardiac output are complex and, considered individually, may suggest seemingly contradictory therapy. For example, the reduction of interstitial fluid is obviously desirable (17). Experimental data in man indicates that if this is achieved, closing volume and shunting are decreased (18). Yet, the use of powerful diuretics to "dry out" the interstitial lung water may not improve oxygenation. An early effect of intravenous furosemide has been shown to be a decrease in pulmonary blood volume and cardiac output with an attendant decrease in oxygen delivery (19). The mechanism of this is probably an acute reduction in plasma volume which might well explain the success of plasma expanders in conjunction with diuretics in pulmonary failure. Plasma expanders will maintain the intravascular volume and cardiac output, reducing ventilation perfusion anomalies, decreasing pulmonary venous admixture, and, incidentally, reducing pulmonary interstitial water by their oncotic action (20, 21). The theoretical danger of the use of oncotically active agents is that if the pulmonary vascular bed is dilated, they may leak into the interstitial space and enhance the net movement of water out of the capillaries (22). This danger can be minimized by regulating fluid administration on the basis of the pulmonary capillary wedge pressure rather than the central venous pressure. It is now possible to place a catheter in the wedge portion at the bedside without difficulty (23). Occasionally, hemodialysis may be justified to remove water (24).

Measures to increase the end expiratory volume of the diseased lung have been so strikingly effective in improving pulmonary function that a test of positive end expiratory pressure (PEEP) should probably be tried in all hypoxic patients. The relationship of the increased lung volume produced to improved lung function is well established (25, 26). More lung is opened up for ventilation with maintained airway positive pressure since the closing volume tends to increase less than functional residual capacity (27). Fairley has pointed out the need to measure gas exchange to assess the effect, in an individual patient, of increasing lung volume with pressure (28). This is necessary since expansion of the lung may encourage the small pulmonary vessels to remain open, while positive airway pressure may have the oposite effect. There is also a price to be paid for this maneuver (PEEP). In both the carefully controlled experiment and clinically, the cardiac output may fall considerably (29, 30). This may occur if the patient is volume-depleted, but it is especially likely to occur in those with an already hypoxic and weakened myocardium. The problem is that while the oxygen content of the arterial blood goes up, cardiac output and the oxygen delivery to the tissues may go down. The patient who goes into severe cardiac decompensation with PEEP can sometimes have the vicious cycle reversed with hyperbaric oxygen. Improved myocardial oxygenation may enable the patient to stand greater airway pressures during and after treatment. A further problem of PEEP may be an increased leakage of air manifested as pneumothorax or mediastinal emphysema (30).

The maintenance of adequate surfactant seems to present a difficult problem, as high tidal volumes increase its consumption (31). Other factors that decrease surfactant production are steroids, hypothermia, and too much oxygen.

Bronchial lavage with 5 to 10 ml of normal saline is now standard respiratory therapy for patients with thick or tenacious secretions. When tenacious secretions become overwhelming, pulmonary lavage (ventilating one lung with normal saline) has produced marked improvement in pulmonary function, especially in patients with status asthmaticus, severe bronchiectasis, or mucoviscidosis (32).

A fall in venous pH can cause a very sharp rise in pulmonary vascular resistance (33). The correction of such an acid-base disturbance may strikingly reduce the resistance. The maintenance of a normal pulmonary vascular resistance during the early stages of shock may be aided by large doses of steroids (34). Forty-micron pore size blood filters for blood administration also appear beneficial (35, 36), as these stop the lung from silting up with the detritus that normally gets through the 170-micron candle filter in the standard blood administration sets. All of these maneuvers have been found to be beneficial in the prevention of "shock lung." Dextran 40® and heparin have been tried especially for microembolism. Except for the now standard use of heparin in fat embolism and the disseminated intravascular coagulopathies, there does not seem to be a strong case for its use in respiratory failure per se. Finally, under certain circumstances, glucagon may decrease pulmonary vascular resistance and decrease shunting (37).

### *Oxygen Delivery*

A difficult question that frequently arises in respiratory failure is the assessment of the adequacy of the delivery of oxygen to the tissues. Anyone with an oxygen tension of less than 50 torr is hypoxic by definition, but all who have dealt with respiratory failure know that patients are frequently tolerant of far lower levels without undue anaerobic metabolism.

The development of an acidosis is frequent in respiratory failure, and this may be metabolic, respiratory, or mixed (38). When oxygenation is inadequate, the clues may lie in performance rather than absolute arterial blood levels. Signs of mental impairment, cardiac arrhythmia, change in the mechanical activity of the myocardium, and loss of renal ability to produce or concentrate urine may be of far greater account. A rise in the serially measured blood lactate level has been demonstrated to be a significant indicator of acute circulatory failure (39). Diminished oxygenation of the venous blood is an excellent index of hypoxia, as the venous oxygen tension is more indicative of the adequacy of oxygen available to cells than the arterial $P_{O_2}$. It is difficult to know what level constitutes damaging hypoxia, as some tissues may be bypassed by A-V shunts or have low flows through them. A jugular venous oxygen tension of less than 20 torr is associated with loss of consciousness (40), but if all compensatory mechanisms are in play, an arterial oxygen tension of 27 torr may be enough to keep the patient awake.

To aid the delivery of oxygen, the heart and circulation must be rendered most effective with adequate blood volume (which may be more than would be expected in health), cardiotonic drugs, such as digoxin, and as much oxygen as can reasonably be supplied. The correction of acidemia to within the 7.32 to 7.54 pH range will ensure both maximum cardiac mechanical efficiency and maximum sensitivity to inotropic drugs.

Oxygen-carrying capacity should be raised if subnormal. Unfortunately, freshly transfused stored blood has a greater than normal binding power with oxygen, leading to a possible "affinity hypoxia." It is very unwise, therefore, to produce an alkalemia in such a patient; the left shift of the oxyhemoglobin dissociation curve and binding of oxygen will be exaggerated. Faster return of the curve to normal has been shown after large doses of methyl-prednisolone sodium succinate (41). The amount of hemoglobin that is optimal to cope with respiratory failure is probably in the normal range. The effect of variable amounts of hemoglobin on oxygen transport have been well discussed by Nunn and Freeman (42). The increased oxygen content of blood when oxygen is supplied at 3 ATA in the hyperbaric chamber can usually be equaled by an additional four grams percent of hemoglobin oxygenated at normal pressures.

## Conclusions

The current methods of treating severe acute respiratory failure have been presented. If these do not produce adequate oxygen delivery to the tissues, the patient will die. The experience presented at this symposium has confirmed that these patients can be kept alive for at least a week on bypass membrane oxygenation and subsequently survive. The question of who should be given this treatment has not yet been answered. The restriction of perfusion to the circumstance when all else has failed will lead to delays which may compromise the survival of the patient. Very high risk situations have to be identified so that patients can be aided before respiratory failure is irreversible. There are already very high risk groups, such as patients in cardiogenic shock or neonates with respiratory distress syndrome, in which the development of a progressive acidemia is accompanied by a very high mortality. In most of the adult pulmonary conditions, the question of when to perfuse is difficult to answer. Unless there is an element of cardiac insufficiency, the methods already available are very successful in reversible lung conditions. The isolated high risk pulmonary groups have a relatively lower mortality than those with a cardiac inadequacy. Patients not improved with optimal therapy after three days of inspired oxygen tension greater than 425 torr to maintain them probably make up one obvious failure group, but there is a need to identify patients who are likely to develop overwhelming hypoxia before they reach this state.

A very pertinent consideration is whether there is sufficient staff to take on what becomes an exercise in constant vigilance, hard work and comprehension. Initially, when an institution takes on such a mode of therapy whole departments may be rendered otherwise ineffective in other, often very important areas. The requirement of a large trained multidisciplinary team with a high sense of commitment cannot be overstressed as a way to success.

## Summary

A review of the current treatment of acute respiratory failure and the problems of oxygen therapy, oxygen consumption, oxygen delivery, and cardiopulmonary function are presented. Except for cardiogenic shock and severe respir-

atory distress syndrome in infants, it is difficult to identify high risk groups who might benefit from early bypass membrane oxygenation. Patients requiring prolonged therapy with high oxygen concentrations may constitute one such group. An adequate trained and committed team is needed.

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# Extracorporeal Oxygenation for Acute Respiratory Insufficiency: Clinical Experience

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## Introduction

Volume controlled respiratory and quality intensive care are still the treatments of choice in the vast majority of patients with acute respiratory insufficiency. Prolonged extracorporeal oxygenation should be considered as an acceptable alternative in patients who are approaching lethal hypoxemia when one has exhausted the conventional therapeutic remedies. By 1977, approximately 66,000 patients a year will die in the United States as a direct result of acute respiratory insufficiency and its complications (1). It is increasingly important to seek new modes of therapy for these patients.

### *Indications for Initiating Extracorporeal Oxygenation*

The decision to initiate extracorporeal oxygenation is largely one of clinical judgment because, unfortunately, no hard data are available to indicate to a clinician when a patient is approaching hopeless respiratory insufficiency and when procrastination with conventional therapy can lead to a worse situation 48 to 72 hours later. We use the following guidelines in making this decision:

1. The arterial oxygen tension must be consistently at or below 40 torr when the patient is receiving maximal respiratory support ($F_{IO_2}$, 1.0; PEEP*, + 8 cm $H_2O$; volume controlled respirator).
2. All pleural fluid accumulations must be removed and any pneumothoraces adequately treated and brought under control.
3. The patient must have had maximum dehydration using diuretics.
4. Evidence of hypoxemic myocardial depression exists.
5. Evidence of hypoxemic CNS depression exists.
6. The summation effect of generalized hypoxemia on the ability of the patient to deal with secondary complications is not known, but in our hands, septicemia, disseminated intravascular coagulation (DIC) and renal failure, in the presence of severe hypoxemia, have contributed to a fatal outcome in many such cases. We consider the appearance of these complications an indication that the body may be losing the fight against generalized hypoxemia.
7. If we are satisfied that the patient is progressing to a moribund state, perfusion is started as soon as this decision is made.

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* Positive end expiratory pressure.

8. The presence of active bleeding (nasopharyngeal, tracheal or gastrointestinal) are contraindications. Postoperative patients following major surgery are safe for perfusion. Neurosurgical postoperative patients should be eliminated specifically because of the risk from bleeding.

9. The pulmonary pathology must be potentially reversible.

The final solution to the problem of patient selection will come when we can more accurately predict the outcome in cases of acute respiratory insufficiency

TABLE I

*Case Material*

| Case No. | Age (yr.) | Diagnosis | Duration of bypass | Comment |
|---|---|---|---|---|
| | | | Venovenous Bypass | |
| 1 | 26 | Viral pneumonia; coma | 26 hours | Successfully taken off bypass; died 3 days later with Pseudomonas septicemia |
| 2 | 16 | Pulmonary fat emboli; coma | 23 hours | Taken off bypass; died 1 day later with cerebral fat emboli |
| 3 | 19 | Traumatic pulmonary contusion | 6.5 days | Taken off bypass; subarachnoid hemorrhage 1 day later due to cerebral trauma, heparin, and thrombocytopenia; died 6 days later |
| 4 | 24 | Viral pneumonia | 36 hours | Died due to viral myocarditis and severe left heart failure while on perfusion |
| 5 | 19 | Viral pneumonia | 58 hours | Taken off bypass; died 9 days later with sudden cardiac arrest of unknown etiology |
| 6 | 3 | Respiratory burn; oxygen toxicity | 12 hours | Died on bypass; intraperitoneal hemorrhage |
| | | | Venoarterial Bypass | |
| 7 | 24 | Traumatic shock lung; lacerated thoracic aorta; fractured pelvis, tibia and fibula | 75 hours | Taken off bypass; long-term survivor |
| 8 | 20 | Chest deformity (polio); aspiration pneumonia; oxygen toxicity (8 days) | 30 hours | Died on bypass; irreversible pulmonary damage and technical failure |
| 9 | 51 | Chicken pox; viral pneumonia; renal shutdown | 60 hours | Died on bypass; hypertensive crisis; blood pressure 240/140 mm Hg; followed by subarachnoid hemorrhage; DIC reversed |
| 10 | 17 | Barbiturate overdose; pulmonary edema; aspiration pneumonia; massive bilateral pneumothoraxes | 9.5 days | Died 2 hours after coming off bypass; pulmonary function improved during first 6 days of perfusion, then failed to improve further; she was removed from bypass although her lungs had not improved as much as was probably necessary; died with respiratory failure |
| 11 | 26 | Amniotic fluid emboli; shock lung; DIC | 27 hours | Died on bypass; uncontrollable DIC |
| 12 | 19 | Pulmonary fat emboli; renal shutdown; coma; fractured femur | 5 days | Survival; DIC reversed; difficult post-perfusion course due to cerebral and renal fat emboli; kidneys and brain returned to normal; tracheo-esophageal fistula |
| 13 | 47 | Overdose; shock lung; meningitis DIC; pulmonary infection; septicemia | 77 hours | Taken off bypass with good respiratory function; DIC reversed; died 12 days later with progressive pulmonary infection, anuria, coma and meningitis |
| 14 | 2 | Postoperative VSD repair and removal of pulmonary artery band; left heart failure and pulmonary consolidation | 11 hours | Died with left heart failure and DIC; the child weighed 10 Kg. |
| 15* | 10 | Postoperative tetralogy; hypoxemia | 44 hours | Survival; rapid improvement in $Pa_{O_2}$ after first 33 hours of perfusion |
| 16 | 56 | Aspiration pneumonia; DIC; septicemia; shock | 6 hours | Died on bypass; uncontrollable DiC and left heart failure |

* Performed in Düsseldorf, West Germany by Hagen Schulte, M.D.

so that action may be taken early, or when the risk of perfusion becomes so small that its application will pass into the category of prophylactic therapy.

### *Clinical Material*

We have used extracorporeal oxygenation in 16 patients to treat acute respiratory insufficiency (2–4). Details of each case are indicated in Table I. The age of the patients has varied from three years to 56 years, with 13 of the 16 patients under 26 years of age. Duration of perfusion has lasted from 6 hours to 9.5 days. Acute pulmonary insufficiency has been the principal lesion in all patients but various degrees of left heart failure were also present in some of the patients.

### *Technique of Perfusion*

Two different methods of cannulation have been used for prolonged extracorporeal oxygenation. A venovenous technique was used in the first six patients as diagrammatically illustrated in Figure 1. In the last ten cases, a venoarterial procedure was employed and is represented in Figure 2. In either procedure both proximal and distal cannulation are made in the vein or the artery of the groin or the neck to prevent distal edema or ischemia resulting from occlusion of a distal vessel. Cannulas are introduced without difficulty in both directions through the same transverse venotomy or arteriotomy. At the conclusion of bypass, the cannulas are removed and the vein or the artery repaired by standard methods. The largest size wire-reinforced vinyl cannulas possible are introduced for venous drainage. These sizes vary from a #32 to a #36 French. The position of the venous cannula is checked by x-ray.

Heparin is used for systemic anticoagulation in all patients. In the first six cases, an initial dose of 300 units per kilogram was administered, followed by

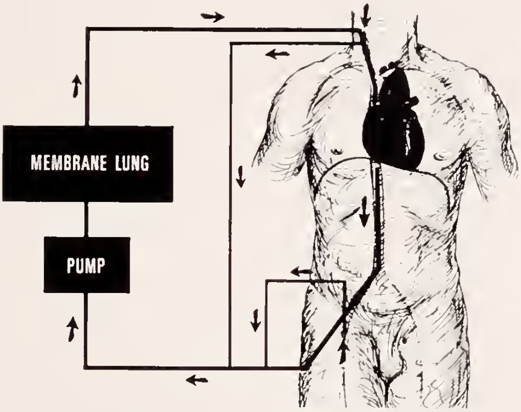

Fig. 1. This diagram illustrates the cannulation technique for venovenous bypass. Blood is removed from the inferior vena cava by way of a cannula introduced into the right common femoral vein. The distal common femoral vein is cannulated as well. Blood is pumped through the membrane oxygenator and the fully saturated blood is returned to the superior vena cava by way of a cannula introduced into the internal jugular vein. The distal internal jugular vein is cannulated as well and contributes to the venous return (Figures 1, 2, and 3 are reproduced with permission from Hill et al: Acute Respiratory Insufficiency, J Thorac Cardiovasc Surg 64: 551, 1972.)

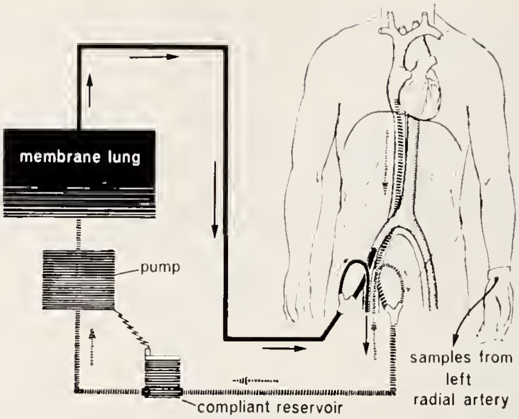

FIG. 2. Venoarterial technique. Venous blood is removed from the right atrium by way of a cannula introduced into the right common femoral vein. The distal common femoral vein is cannulated as well. The venous blood is pumped through the membrane oxygenator and returned to the body by way of retrograde perfusion through the right common femoral artery. The distal femoral artery is perfused as well. The groin incision is closed while the patient is on perfusion. Arterial samples are drawn from the left or right radial artery. This insures sampling at a point where the potentially lowest levels of $Pa_{O_2}$ will be found. Deceptively high values from the lower parts of the body are thus avoided.

100 units per kilogram thereafter. This produced a Lee-White clotting time of infinity in most instances. In the last ten cases we have reduced the administration of heparin. The patient was given 100 units of heparin per kilogram initially and then a ten percent heparin in saline solution is continuously infused with a calibrated pump into the heart-lung machine at a rate necessary to maintain a Lee-White clotting time of 25–40 minutes. The dose necessary to maintain this range varies between 15–45 units per kilogram per hour with dosage requirement depending mainly upon the amount of urinary output. The level of clotting activity is measured by means of the "activated clotting time" technique (5). This technique provides an accurate result within four minutes and has been proven very satisfactory in the management of continuous heparin administration.

Following the initiation of extracorporeal oxygenation, the $F_{IO_2}$ of the respirator is reduced to 0.6 or 0.7, and its tidal volume is reduced to lower levels so that the levels of plateau pressure in the trachea are not excessively high. All samples for blood gases are drawn from the right or left radial artery because sampling from this site insures that one will monitor the lowest possible level of $Pa_{O_2}$ in the arch of the aorta when employing a retrograde perfusion technique.

Cannulation is performed under local anesthesia. The patients are kept awake throughout the perfusion with liberal doses of morphine used intravenously to insure relative comfort. Several of our patients were in a coma or semicomatose condition before and during perfusion so that no sedation was necessary.

The Bramson membrane heart-lung machine (6.0 $M^2$ membrane surface) was used in all perfusions (6). This machine has an integral heat exchanger in the oxygenator and also a servo-mechanism whereby venous return flow automatically controls the pump speed and returns oxygenated blood to the patient at precisely the same rate. All perfusions were conducted at 37° C.

## Results

*Venovenous technique.* Six patients were treated with venovenous technique and four of them were taken off perfusion with satisfactory pulmonary function enabling them to maintain an adequate level of oxygenation until they died from nonpulmonary causes. There were no long-term survivors.

*Venoarterial technique.* Ten patients were perfused using venoarterial technique and five of them were taken off perfusion with satisfactory pulmonary function. Three were long-term survivors. (See Table I for details).

### *Oxygenation and Cardiopulmonary Dynamics*

Figure 3 indicates the $Pa_{O_2}$ both for the venovenous and the venoarterial groups of patients before, during, and after perfusion. Satisfactory step-up in the systemic $Pa_{O_2}$ was noted in patients using both techniques; however, it was higher when using the venoarterial bypass procedure. Venovenous technique produced 55 percent of the total oxygen requirements versus 65 percent of the

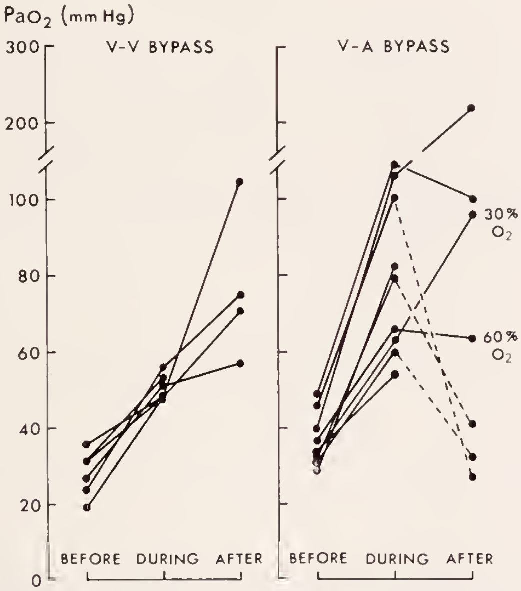

FIG. 3. This graph indicates the $Pa_{O_2}$ in all patients in whom satisfactory data were obtained before, during and after perfusion. To make the samples drawn before and after perfusion comparable, the patient was given 100 percent oxygen through the respirator during sampling except when otherwise indicated. The respirator settings during perfusion were an $F_{IO_2}$ of 0.5 or 0.6 and reduced tidal volumes. The dotted line denotes the $Pa_{O_2}$ values of three patients who were taken off bypass on 100 percent oxygen but died within 30 minutes. It is evident that in these patients no satisfactory improvement of the pulmonary function was obtained. The perfusions were discontinued for technical reasons or because it was thought the lungs had reached the point of irreversible damage.

TABLE II

*Cardiopulmonary Hemodynamics during Perfusion*

*(RANGE & AVERAGE)*

| | Venovenous Bypass | Venoarterial Bypass |
|---|---|---|
| Increase $Pa_{O_2}$ (mmHg) | 22 | 57 |
| | (13-31) | (32-95) |
| Cardiac Output through Oxygenator (%) | 48 | 55 |
| | (31-65) | (50-68) |
| $O_2$ Exchange (%) | 55 | 65 |
| | (41, 70) | (34-80) |
| $CO_2$ Exchange (%) | 38 | 61 |
| | (37, 39) | (44-80) |
| Decrease PAP (mmHg) | 0, 1 | 16 |
| | | (10-23) |

total when venoarterial technique was used. Similarly, the carbon dioxide exchange using venovenous technique was 38 percent while 61 percent was recorded with the venoarterial system. Venoarterial bypass flow constituted a higher percentage of the total systemic flow than that obtained with venovenous technique, i.e., 55 percent versus 48 percent. However, the 55 percent average of the total systemic flow obtained by venoarterial technique is unrealistically low due to the fact that technical reasons caused several patients to have low bypass flows. Our technique is now perfected to a point enabling us to obtain 65–70 percent of the total systemic flow most of the time. The primary advantage of the venoarterial over the venovenous technique is its effect on the pulmonary artery pressure. The venoarterial method drops pulmonary artery pressure by 16 mm of Hg whereas venovenous technique had no effect whatsoever. These data are summarized in Table II.

### *Hematologic Data*

Hemolysis during long-term perfusion is not a problem. Figure 4 indicates the levels of plasma-free hemoglobin obtained in all 16 patients with perfusions of up to 200 hours in duration. The majority of readings are below a level of 40 mg percent. Higher points occurred as a result of blood transfusion reactions and these patients lowered their plasma-free hemoglobin by the following day as hemoglobin was eliminated by the kidneys and the reticuloendothelial system.

The most prominent hematologic effect of bypass is a decrease in the level of platelets and this phenomenon causes some problems (Fig. 5). Over half the patients had a platelet count of less than 100,000 before bypass began. However, once bypass was begun, circulating platelets dropped between 40 and 60 percent in all patients. In some cases, dangerously low platelet levels were encountered. Platelet transfusions were administered to approximately one half of the patients in an effort to bring the level of the circulating platelets above

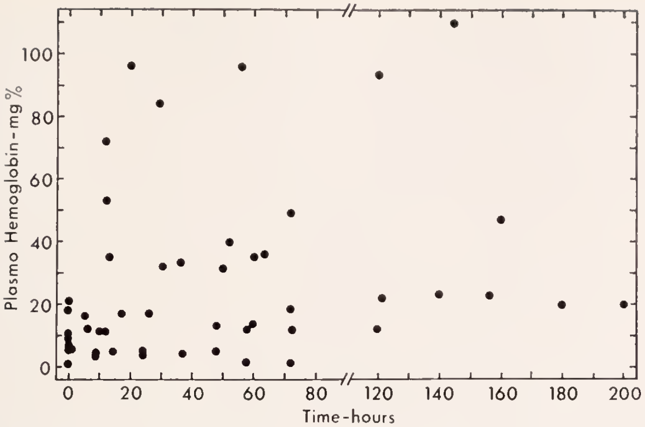

FIG. 4. The plasma free hemoglobin in all patients is plotted here against hours of perfusion.

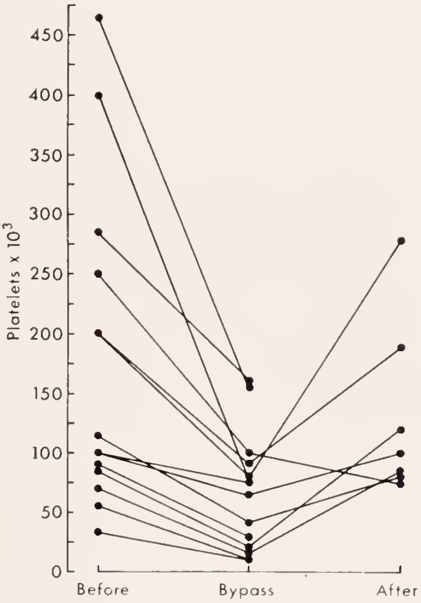

FIG. 5. This graph indicates the platelet counts before, during, and after perfusion.

30,000. These transfusions promptly raised the platelet counts to more acceptable levels; however, it was always a short-lived phenomenon and after four to six hours the platelets would once again be at a very low level. The average curve of the number of circulating platelets in patients who received platelet transfusions and those who did not are indicated in Figure 6, providing evidence that platelet transfusions have no constant effect on the level of the circulating platelets. Immediately following the termination of bypass in all patients, platelet counts increased substantially.

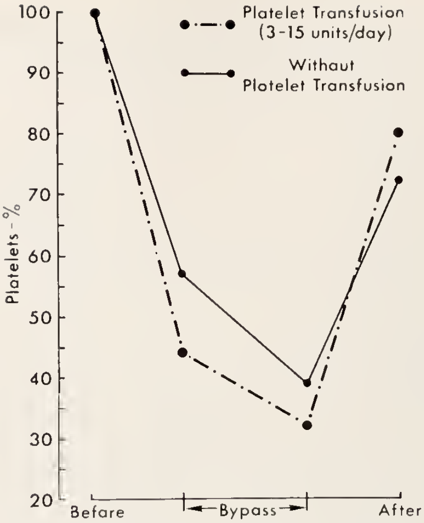

FIG. 6. This graph indicates the average platelet counts of patients who either received or did not receive platelet transfusions. In the patients who received platelet transfusions, the overall level of circulating platelets during perfusion did not rise. Following perfusion both groups of patients responded satisfactorily with increased circulating platelets.

The level of circulating fibrinogen was not altered by extracorporeal circulation. Five patients had clinical and laboratory evidence of DIC before bypass and three of these patients had completely reversed this picture within 24 hours from initiation of bypass. In the other two patients we were unsuccessful in influencing the course of the DIC syndrome. However, one of these patients had amnionic fluid emboli which is an extremely strong stimulus to DIC and the other patient had severe left heart failure and septicemia as a triggering mechanism. No patients developed DIC while on bypass.

Thrombogenesis within the membrane oxygenator was not observed during perfusion. After each clinical case, the artificial lung was disassembled layer by layer and examined for any evidence of clots, but none could be found.

The control of bleeding is largely a matter of surgical technique and careful use of heparin. Using careful surgical technique and electrocautery, bleeding from the incision is usually absent or minimal. We had one fatal hemorrhage in the first six patients in whom we used large heparin doses with essentially a Lee-White clotting time of infinity (Case 6). We then changed to a Lee-White clotting time of 25 to 40 minutes and have since had no serious bleeding complications related to heparin.

## Discussion

We believe the venoarterial technique is superior to the venovenous technique in achieving better physiological conditions during long-term bypass. Advantages of venoarterial technique appear to be 1) a higher step-up in $Pa_{O_2}$; 2) an increased percentage of systemic flow diverted through the extracorporeal circuit; 3) a larger proportion of the total oxygen requirements supplied by the artificial lung; 4) a reduction in the mean pulmonary artery pressure which is conducive to healing of the lungs; and 5) a greater reduction in dangerous $F_{IO_2}$ and unphysiological high plateau respiratory pressures, often produced by a volume controlled respirator, than venovenous technique permits. Moreover, this is consistent with our philosophy of using extracorporeal perfusion not only to buy time for the lungs to heal but also to provide an improved environment for that healing process.

When and whom to bypass continues to be the foremost problem in utilizing extracorporeal oxygenation for respiratory insufficiency. Opinions vary widely, from the ultraconservative to the unrealistically liberal, as to criteria for the correct selection of patients in this procedure. When such a debate exists in medicine, it usually indicates that insufficient data are available in the field. This is the case with acute pulmonary disease, where various proponents of different approaches must base decisions on inadequate knowledge of the pathogenesis, natural course, and effect of each therapy on the natural course of the disease. The existing situation is as follows: Although the majority of patients with varying degrees of acute respiratory insufficiency can be managed successfully at most modern pulmonary centers, a large number of patients with severe forms of respiratory insufficiency die. We do not know what turns a mild degree of pulmonary insufficiency into severe respiratory embarrassment, nor can we predict, by any measurable parameter, which cases will be so transformed. We do not know why fatal pathology develops in the lungs of some patients at an accelerating rate. Physicians working in intensive care units can often cite patients who overcame what could be called impossible odds and recovered satisfactory pulmonary function; but more commonly, these patients die. There are no criteria by which to classify degrees of pulmonary insufficiency except in such terms as the $P_{aO_2}$ and the A-a gradient. Respiratory parameters in no way define the pulmonary pathology present, although this will eventually determine the outcome of the disease. We cannot yet formulate an accurate prognosis for an individual patient before the patient is clearly moribund and past help from any form of therapy. On the other hand, prolonged extracorporeal oxygenation is a largely untested procedure. There have been instances in which the pulmonary failure has been completely reversed by perfusion; however, direct complications and deaths have also been caused by it (7). To make the decision more difficult, we have also observed that results of perfusion have been better when, out of necessity under the threat of lethal hypoxemia, patients were placed on bypass early in their disease during the stage of congestion and inflammation rather than later when hyaline membranes, bacterial infection, microabscesses, and the beginning of pulmonary fibrosis were present.

The clinician, then, is faced with the problem of continuing conventional treatment of a patient who he believes will be hypoxic and dying in three or four days, in the hope that the patient will survive this approach; or, alternatively, trying to alter the course of the disease by using extracorporeal oxygenation early.

Until extracorporeal circulation can be made a procedure without significant risk, we will continue to use the guidelines as outlined in the beginning of this paper. The single most important element in making prolonged perfusion a safe procedure is the elimination of any risk of bleeding in the patient (8).

## Summary

Sixteen patients have undergone prolonged extracorporeal oxygenation for acute respiratory insufficiency. Nine of the 16 patients were taken off perfusion with satisfactory pulmonary function. Three were long-term survivors. The technique, cardiopulmonary dynamics, and hematologic effects of perfusion have been described. The criteria for the selection of patients have been defined.

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# Prolonged Extracorporeal Oxygenation: Some Special Problems

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The most immediate goals and prerequisites for success in prolonged extracorporeal oxygenation are: 1) Establishing a satisfactory blood bypass flow rate producing an improved and adequate systemic oxygen tension level; 2) Substantially reducing the settings of the respirator ($FIO_2$, tidal volume, PEEP, and MIP) to physiologically tolerable levels so that the lungs are not damaged by the respirator at a faster rate than they can heal; and 3) Control of hematologic phenomena associated with extracorporeal oxygenation.

These three factors have been extensively reviewed in recent publications (1–4). There are, naturally, other important facets of this procedure that can determine success or failure. We have learned a number of logistic and management principles in our series of 14 perfusions for severe respiratory insufficiency. The purpose of this paper is to report on our current management techniques and the criteria for decisions which must be made in the course of this new therapy for respiratory insufficiency.

## *Selection of Patients*

Opinions vary widely, from the ultraconservative to the unrealistically liberal, as to the criteria for the correct selection of patients for this procedure. Whenever a debate such as this exists in medicine, it is usually the result of decision-making being forced on clinicians in fields where insufficient data are available. One must balance results which can run from miraculous recovery to death, using a new controversial method, against the known and usually unacceptable results of current management. This type of debate is presently proceeding on a national scale concerning elective aorto-coronary vein bypass procedure for stable angina.

In acute pulmonary disease the various proponents of different approaches are having to base their decisions on inadequate knowledge of the pathogenesis, of the natural course, and of how each therapy will affect the natural course of the disease. The following dilemma exists: The majority of patients with varying degrees of acute respiratory insufficiency can be managed successfully at most modern pulmonary centers. A large number of patients with severe forms of respiratory insufficiency die. It is forecast that in 1977, there will be 86,000 deaths in the United States resulting from acute respiratory

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insufficiency (5). We do not know what turns a mild degree of pulmonary insufficiency into severe respiratory embarrassment. Nor can we, by any measurable parameter, predict which cases will be so transformed. Why does an accelerated rate of fatal pathology develop in the lungs of some patients? Figure 1 shows pulmonary fibrosis within nine days of the initial onset of pulmonary disease. Was this the result of the disease or the treatment ($O_2$, PEEP, etc)? All physicians working in intensive care units have seen patients who overcame what could be called impossible odds and recovered satisfactory pulmonary function. More commonly, these patients die. There are no criteria by which to classify degrees of pulmonary insufficiency except in terms of the

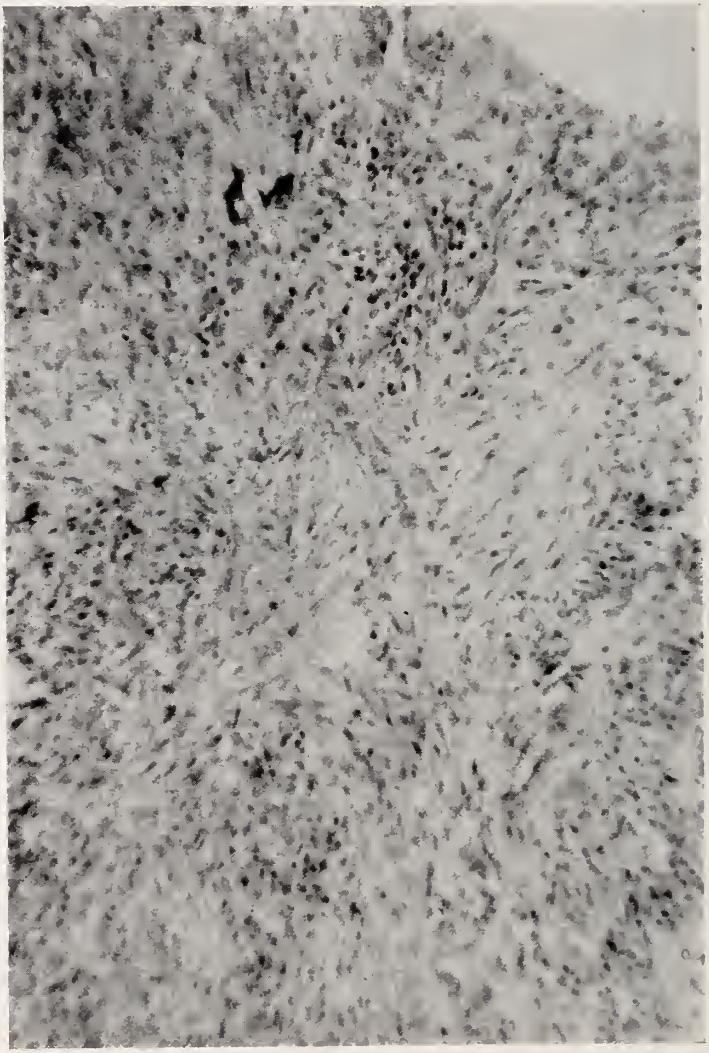


FIG. 1. Lung section showing pulmonary fibrosis already present 9 days after the onset of pulmonary viral pneumonia (H & E 10×).

$Pa_{O_2}$ and the A-a gradient. These in no way define the pulmonary pathology present which will eventually determine the outcome. We cannot take an individual patient and classify him or her so that the prognosis can be established before he or she passes into a moribund condition, at which time any form of treatment would be useless. However, I believe that clinicians, experienced in dealing with severe respiratory insufficiency, 80 to 90 percent of the time can predict the ultimate outcome some days before the final event. More precise data are badly needed.

On the other hand, prolonged extracorporeal oxygenation is a largely untested procedure. There have been some instances where the pulmonary status has been completely reversed by perfusion. There have also been direct complications and deaths caused by it. To make the decision more difficult, we have also observed that the results of perfusion have been better when out of necessity, due to lethal hypoxemia, patients were placed on bypass early in their disease, during the stage of congestion and inflammation, rather than late in their disease when hyaline membranes, bacterial infection, and microabscesses are present.

The clinician is then faced with the problem of either continuing conventional treatment of what he believes may be a hypoxic, dying patient in three or four days in the hope, even if it is small, that the patient will survive this approach; or alternatively, of trying to alter the course of the disease by using extracorporeal oxygenation early.

With accumulating experience, we are slowly moving into the latter approach. We now consider the following factors:

1. The arterial oxygen tension must be consistently below 40 torr while the patient is receiving maximal respiratory support ($F_{IO_2}$ 1.0; PEEP + 8; volume-controlled respirator).
2. All pleural fluid accumulations and pneumothoraces must have been removed or be under control and pulmonary parenchyma must have had maximal dehydration using diuretics.
3. Evidence of hypoxemic myocardial depression.
4. Evidence of hypoxemic CNS depression.
5. The summation effect of generalized hypoxemia on the patient's ability to deal with secondary complications is not known. Septicemia, DIC, and renal failure in the presence of severe hypoxemia in our hands has contributed to a fatal outcome in most situations. We consider the appearance of these complications as an indication that the body may be slowly losing the fight against generalized hypoxemia.
6. If we are satisfied that the patient is progressing to a moribund state, perfusion is started early rather than late.
7. The presence of active bleeding (nasopharyngeal, tracheal, gastrointestinal) are contraindications. Postoperative patients following major surgery are safe for perfusion. Neurosurgical postoperative patients should be eliminated, specifically because of the risk from bleeding.
8. The patient must have a potentially reversible pulmonary pathology.

The final solution to the problem of patient selection will come when we can accurately predict the outcome in cases of acute respiratory insufficiency so that any action may be taken early; or when the risk of perfusion becomes so small that its application will pass into the category of prophylactic therapy. I believe the latter will occur before the former.

### *Assessment of Pulmonary Function during Perfusion*

Under extracorporeal support, how long does it take the pulmonary pathology to improve? The answer is completely unknown. We have seen striking and rapidly progressive improvement in pulmonary function following two to three days of extracorporeal oxygenation. We have also seen no improvement at all. It is important to recognize changes in the pulmonary status so that the duration of the perfusion can be more carefully planned. What guidelines can be used to determine improvement or deterioration in pulmonary function?

The chest roentgenogram is not accurate in reflecting changes in pulmonary function. When pulmonary function improves on bypass, the x-ray may be the same or improved. Thus, we cannot rely on the x-rays but check them daily, or more often, for the presence of pneumothorax, pleural fluid or atelectasis, which requires specific treatment.

The A-a gradient has been the most useful tool for assessing pulmonary improvement. The $F_{IO_2}$ is 1.0 in all patients before perfusion. Once a stable bypass is obtained, the $F_{IO_2}$ is reduced to 0.4 to 0.6. The tidal volume is also decreased. Periodically, the $F_{IO_2}$ is increased to 1.0 in order to measure the A-a gradient. If the gradient is decreasing, the pulmonary function is improving. This measurement has been the best means of assessing the pulmonary status during perfusion.

The pulmonary compliance has been useful in reflecting changes in pulmonary pathology. Patients who have not shown improvement in function as measured by the A-a gradient have also not shown improvement in the pulmonary compliance.

Although we have not used it extensively yet, a decrease in the pulmonary vascular resistance will probably reflect improvement in pulmonary function. This parameter can be measured easily, quickly and accurately, and hopefully will be a good reflection of changes occurring in the lungs.

### *Discontinuing Bypass*

Our criteria for deciding whether to discontinue perfusion have been as follows:

The volume-controlled respirator is adjusted to safe levels that will not produce additional lung injury ($F_{IO_2}$ less than 0.6, MIP less than 50 cm $H_2O$).

The bypass is continued until the $Pa_{O_2}$ is stabilized at around 80 torr. The bypass flow is then reduced in steps as long as the $Pa_{O_2}$ remains satisfactory, indicating that the patient's lungs are oxygenating the additional flow through them. This procedure takes three to six hours. If we are not successful in coming completely off perfusion, we return to the highest flow we can get. If we are successful in discontinuing perfusion, we wait 30 minutes. We then remove the cannulae if pulmonary function has remained satisfactory. During any waiting period before the cannulae are removed, the blood in the heart lung machine is continuously recirculated so that no clotting will occur.

### *Blood Volume and Fluids*

In the absence of overt bleeding requiring immediate replacement, there is still a necessity for some volume replacement daily. Blood sampling for various studies require up to 250 ml of blood per day. If blood volume replacement is made chronically with whole blood, there is a tendency for the hematocrit to fall. We, therefore, usually use packed red cells for routine replacement. Most blood volume replacement is required in the first 12 to 18 hours. Thereafter, only small amounts are usually necessary unless there is bleeding. Sometimes several days may go by without requiring any blood transfusions. All blood is transfused through a Swank blood filter.

Human albumin is administered daily at a rate sufficient to maintain the serum proteins at a physiological level so that the oncotic pressure stays in the normal range.

Normal daily fluid requirements are given at a minimum to keep the patient on the dehydrated side. The serum osmolality is kept between 325 to 350 mOsm per liter.

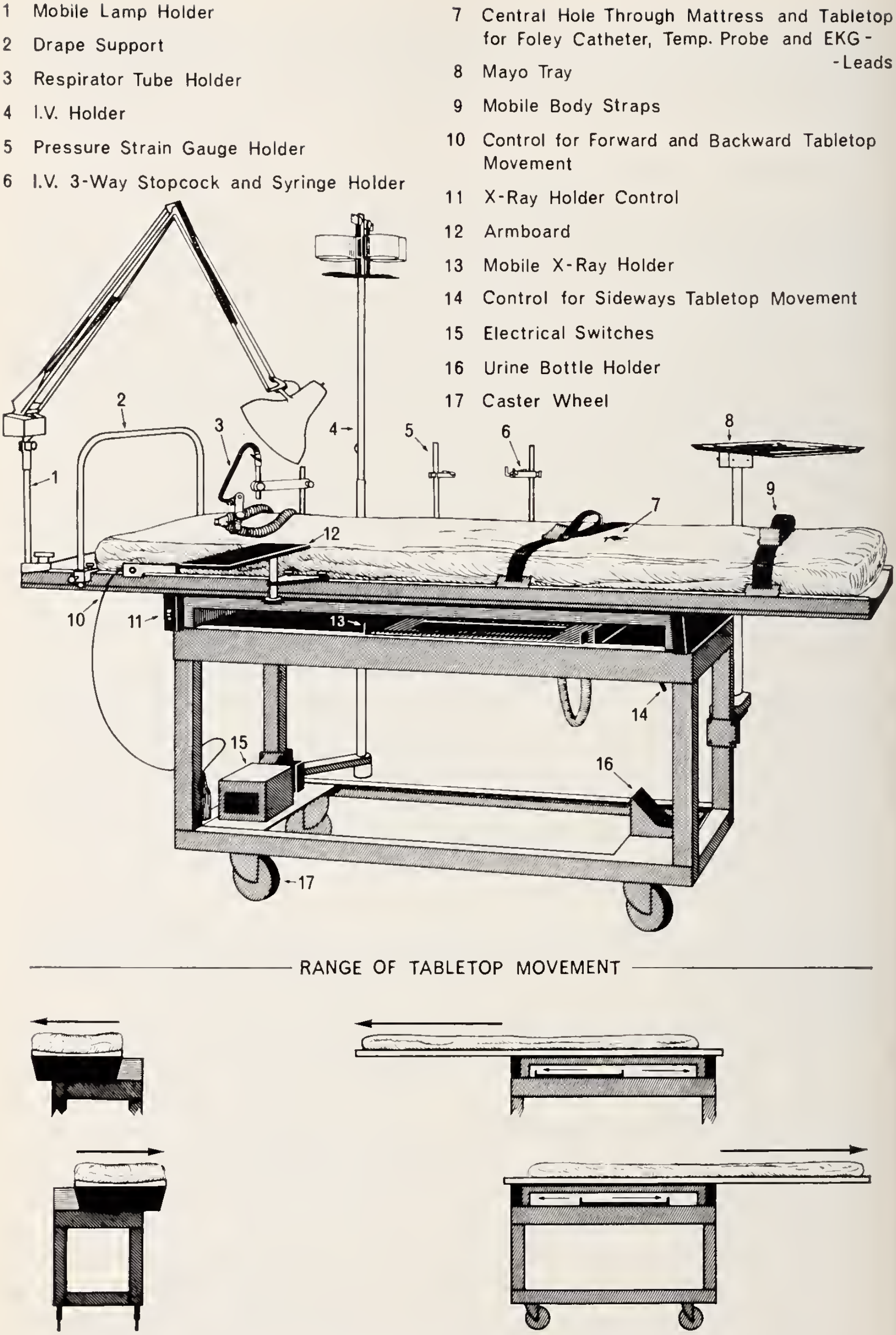
Very often a patient will have oliguria or anuria before perfusion begins due to concomitant renal failure. In these instances, dialysis will have to be performed during perfusion. This is technically very easy to perform since a high pressure cannula can be connected to the aortic perfusion line and to the dialysis machine. Once on dialysis, care must be taken to remove fluid slowly or there will be a large swing in the arterial blood pressure.

### *Logistics of Management*

Patients with acute respiratory insufficiency present certain problems of management. The addition of extracorporeal oxygenation can only compound this problem.

The most pressing overall problem during long term perfusion is a desire to do meaningful investigative studies and, at the same time, to attend to the moment to moment needs of this type of procedure. We have made substantial progress in the logistic management of these patients so that they can be managed smoothly and efficiently. The solution to this problem is automation of all things that can be predicted and prearranged. To this end, the following procedures are used.

1. All standard cardiopulmonary data and pulmonary mechanics data are sensed, stored, recorded and displayed on either a TV screen or in hard copy, using an on-line digital computer (IBM 1800). Thirty-one different parameters are collected (4, 6). The nurses record only special isolated pieces of information.


1 Mobile Lamp Holder
2 Drape Support
3 Respirator Tube Holder
4 I.V. Holder
5 Pressure Strain Gauge Holder
6 I.V. 3-Way Stopcock and Syringe Holder
7 Central Hole Through Mattress and Tabletop for Foley Catheter, Temp. Probe and EKG-Leads
8 Mayo Tray
9 Mobile Body Straps
10 Control for Forward and Backward Tabletop Movement
11 X-Ray Holder Control
12 Armboard
13 Mobile X-Ray Holder
14 Control for Sideways Tabletop Movement
15 Electrical Switches
16 Urine Bottle Holder
17 Caster Wheel
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16
17
RANGE OF TABLETOP MOVEMENT

Fig. 2. Intensive care treatment table.

2. There are standard orders for all hematologic and metabolic blood samples, cultures, urine samples, and metabolic measurements that are collected at preset times. The results are entered into a computer in the clinical lab and are immediately available for use at the bedside by either TV screen or hard copy. Up-to-date 24 hour graph data are always available at the bedside on the TV screen therefore eliminating the need to search charts for information.
3. The nursing responsibilities are separated. One nurse looks after the nursing needs of the patient, that is, suctioning, turning, wound care, respiratory therapy, caring for the pressure sensing lines, and talking to the patient. A second nurse or technician manages all fluid administration, drug administration, heparin control, and sample taking. All drugs and fluids are administered by way of the extracorporeal circuit well away from the patient. This helps to keep the activity level around the patient at a minimum. All decisions in the management of the patient are made and enacted by one or two senior staff and not by a committee of doctors representing every organ in the body.
4. The construction in our machine shop of a special diagnostic and treatment table has greatly facilitated the care of these patients and those other severely ill patients not necessarily requiring extracorporeal oxygenation (Fig. 2). The table has the following features that make it useful for this purpose:
    a. A movable fluoroscopic top for positioning of intracardiac catheters, pacemakers and endobronchial irrigation and suctioning.
    b. An x-ray cassette under the table.
    c. A table narrow enough to perform surgery on, easily.
    d. A full set of accessory equipment that attaches to the bed for use in the care of acutely ill patients.

## Summary

The overall success of prolonged extracorporeal oxygenation will depend upon our ability to treat the whole patient, to pay attention to small details and not permit our efforts to be only lung-oriented. Patients who develop acute respiratory insufficiency are often subject to, or already suffer from concomitant neurologic dysfunction, disseminated intravascular coagulation, renal failure, stress ulcers or septicemia. The successful management and, more important, prevention of these complications will contribute heavily toward success.

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# Respiratory Gas Exchange with Spiral Coil Membrane Lungs: Total Cardiopulmonary Bypass in Adult Sheep and Clinical Experience with Long-Term Perfusion in Adult Patients

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Clinical perfusion time with disc and bubble oxygenators is limited by their progressive destruction of the formed elements of blood and denaturation of plasma proteins (1). These deleterious effects preclude long-term support of the patient who cannot maintain his circulation prior to or after cardiopulmonary bypass. Recently developed, commercially available disposable membrane lungs now offer a practical alternative to bubble and disc oxygenators. This paper will summarize our recent experiences with large surface area (2.5 and 5 $M^2$) Kolobow spiral coil membrane lungs, both for short-term total cardiopulmonary bypass in adult sheep and for long-term partial perfusions of patients in terminal, acute respiratory failure.

## Methods and Materials

### *Spiral Coil Membrane Lung*

A series of 0.4 to 5 $M^2$ spiral coil membrane lungs (Sci-Med, Inc.; and Becton Dickinson) are shown in Figure 1. The gas exchange characteristics of this lung have been described by Kolobow (2). In the 2.5 and 5 $M^2$ lungs, humidified oxygen is sucked through inlet ports of a three- to five-compartment sealed silicone membrane envelope, tightly wound around a spool. Blood flows in thin layers around the spiral coiled silicone membrane. In contrast to other membrane lungs, there is no danger of oxygen emboli through a membrane pinhole since the gas phase is at a lower pressure. The gas circuitry is illustrated in Figure 2. A silicone reservoir bag with a 1 mm hole is inserted in the oxygen delivery tubing to allow equilibration to ambient pressure.

Membrane lungs are vacuum drop tested for pinholes prior to use. For priming the membrane lungs, the pressure in the gas phase is set at a constant minus 250 torr. During perfusion the pressure in the gas phase is cycled ten times per minute between minus 150 and minus 250 torr. Oxygen transfer to blood is enhanced by mixing at the boundary layer caused by the roughness of the 4 to 5 mil (0.1 to 0.12 mm) silicone membrane, cast on a supporting knit fabric, and by intermittent pulsation of the gas phase suction source.

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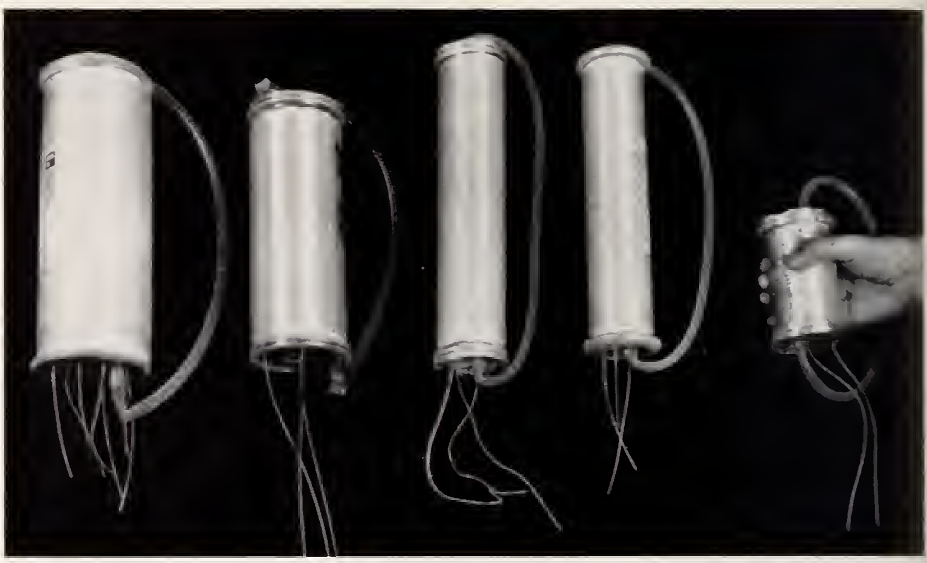

FIG. 1. Spiral Coil Membrane Lungs (0.4; 1; 1.5; 2.5; 5 $M^2$).

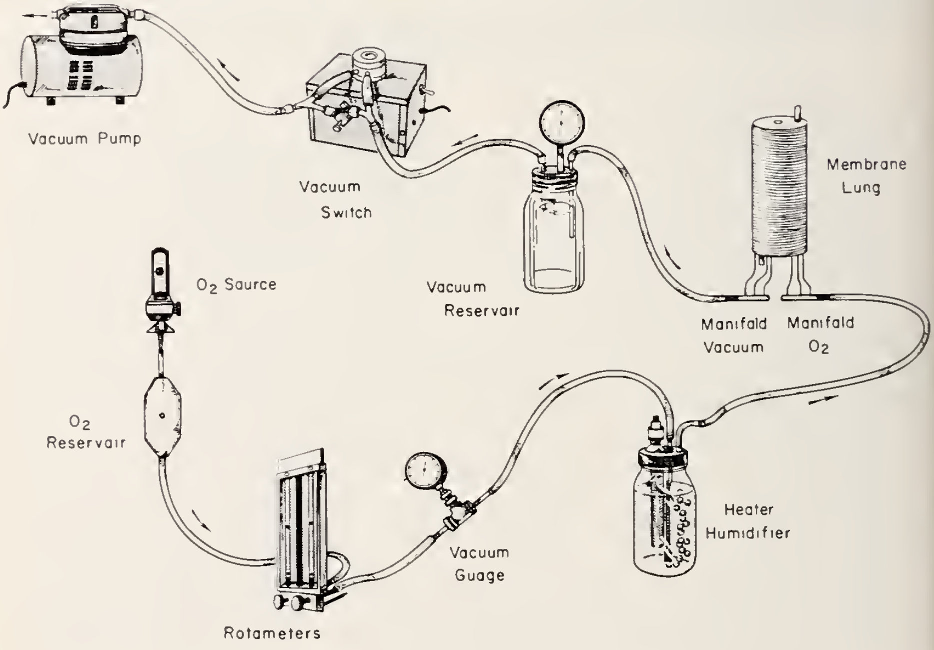
Vacuum Pump
Vacuum Switch
Vacuum Reservair
Membrane Lung
O2 Saurce
Manifald Vacuum
Manifald O2
O2 Reservair
Heater Humidifier
Vacuum Guage
Rotameters

FIG. 2. Membrane lung gas phase circuit.

### *Extracorporeal Circuits*

The extracorporeal circuit for human perfusion is a modification of the closed fixed-prime-volume circuit described by Kolobow (3). Silicone rubber, a silicone-fluid-coated polycarbonate spool, oximeter cells, tetrafluoroethylene plastic connectors, and segmented polyurethane are the only blood-contacting materials. These are all relatively nonthrombogenic materials (4). No free blood-gas interface occurs anywhere in the circuit.

A Sarns precisely nonocclusive roller pump (Model 6202) is used with a specially fabricated fiberglass-reinforced segmented polyurethane pump chamber (20.8 mm I.D.; approximately 100 ml/rev). Thin-walled polyurethane cannulae (fabricated by Raymond E. Johnson), spring-reinforced to prevent kinking, are used to provide maximum flow with low pressure drops. Cannula sizes and flows are seen in Table I and priming volumes are given in Table II. Silicone rubber conduits (½″ I.D.) are fabricated with "T" tubes (⅛″ I.D.; ¼″ O.D.) for pressure measurements, sampling, and infusion.

The circuit for total cardiopulmonary bypass with parallel membrane lungs in adult sheep is illustrated in Figure 3. Blood from the vena cavae is gravity drained (1 to 1.4 M) through two parallel 2.5 $M^2$ membrane lungs into the reservoir bag, nonocclusively pumped through the bubble trap and returned through the carotid artery to the brachiocephalic trunk. In several experiments, a Travenol (Model 5M0335) heat exchanger has been interposed before the bubble trap. No blood recirculation is used.

The circuit for human venovenous perfusion (Fig. 4) and total bypass with membrane lungs in series differs from Figure 3 by placing the membrane lungs after the blood pump. Blood is gravity drained (0.7 to 1.4 M) from the

TABLE I

*Extracorporeal Blood Flow and Cannula Size*

| | 47 Kg Human Venovenous Perfusion | 45 Kg Sheep Total Bypass |
|---|---|---|
| Blood | 6 L/min | 6.8 L/min |
| Flow per Kg | 127 ml/min | 155 ml/min |
| Venous Drainage Cannula | | |
| I.D. | 9.5 mm | 7.8 mm |
| O.D. | 10.4 mm | 8.3 mm |

TABLE II

*Prime Volume of Spiral Coil Membrane Lungs*

| Membrane Lung Size | Prime Volume |
|---|---|
| 0.4 $M^2$ | 65 ml |
| 1.5 $M^2$ | 170 ml |
| 2.5 $M^2$ | 260 ml |
| 3.5 $M^2$ | 370 ml |
| 5.0 $M^2$ | 490 ml |
| **Circuit & Lungs** | **Prime Volume** |
| 2.5 $M^2$ × 2; parallel | 1250 ml |
| 5.0 $M^2$ × 2; parallel | 2500 ml |

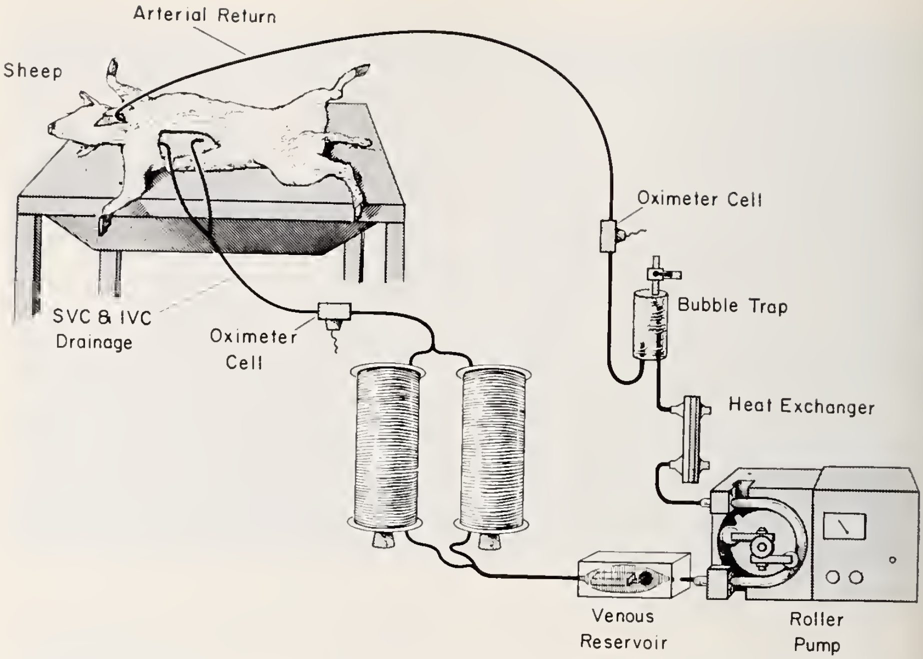
Arterial Return
Sheep
Oximeter Cell
Bubble Trap
SVC & IVC Drainage
Oximeter Cell
Heat Exchanger
Venous Reservoir
Roller Pump

FIG. 3. Cardiopulmonary bypass system with gravity drainage through two parallel spiral coil membrane lungs.

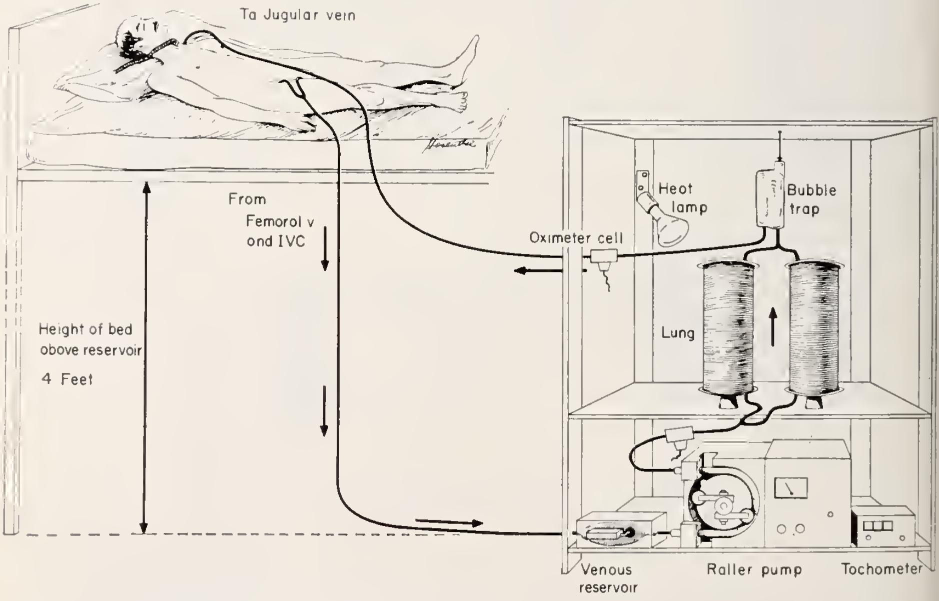
To Jugular vein
From Femoral v and IVC
Heat lamp
Bubble trap
Oximeter cell
Height of bed above reservoir
4 Feet
Lung
Venous reservoir
Roller pump
Tachometer

FIG. 4. Clinical extracorporeal perfusion system. Reproduced with permission from Trans Am Soc Artif Int Organs 18: 553, 1972.

inferior vena cava (IVC) to the venous reservoir bag and pumped through the membrane lungs and bubble trap to the return cannula in the internal jugular vein. Four adult patients and one six-year-old child have been perfused and reported recently by Zapol (5). One perfusion in these patients was venoarterial and the other four were venovenous perfusions.

Several electronic controls are used to monitor the circuit. Venous drainage is controlled by a volume-sensing microswitch on the venous reservoir bag. If venous return diminishes, the reservoir bag collapses and the microswitch automatically stops the roller pump until the reservoir bag reexpands. This limits the suction applied to the venous blood. An electronic high-pressure cut-off switch stops the pump if return line pressure exceeds 350 torr.

To minimize heat loss, the extracorporeal apparatus used for patient perfusions is enclosed in a vinyl canopy and heated with warm air to 40°C.

### *Measurements and Monitoring*

Membrane lung gas exchange is calculated from blood flows and oxygen content differences (Van Slyke). The $CO_2$ concentration of membrane lung effluent gas is determined by an infrared $CO_2$ analyzer (Beckman Model LB-1) at known gas flow rates and $CO_2$ removal rate calculated. Blood oxygen saturation differences across the membrane lungs are continuously measured with a reflectance oximeter (6). Additional blood or plasma determinations include: pH, $Po_2$, $Pco_2$, hematocrit, platelets (phase contrast), sodium, and potassium.

Pressure differences ($\Delta$P lung) are recorded continuously across the membrane lung to monitor membrane lung resistance and across the nonocclusive pump ($\Delta$P pump) with differential transducers (Hewlett Packard 267BC) to compute blood flow and monitor circuit resistance. Blood flow is also monitored with a Nycotron flowmeter with ⅜″ cannulating probe. Central venous pressure, arterial pressure, EKG, esophageal temperature, and urine output are continuously monitored. Patient pulmonary artery and capillary wedge pressures, and mixed venous blood oxygen contents are monitored via a Swan-Ganz catheter. Cardiogreen dye is injected into the pulmonary artery for cardiac output determination (Beckman Cardiodensitometer Model 350127).

### *Total Cardiopulmonary Bypass*

Adult female Hampshire 45 to 88 Kg sheep are anesthetized with IV sodium pentobarbital (30 mg/Kg), intubated, and ventilated with 50 percent $O_2$. Diphemanil methylsulfate (0.5 mg/Kg) is given intravenously to decrease gastric secretions and curare or gallamine are used for muscle relaxation. After a clean right thoracotomy using electrocautery, cannulae (Table I) with sideholes are inserted into the superior and inferior vena cavae via the right atrium. The internal carotid artery is isolated and a cannula (7.6 mm I.D.; 8.1 mm O.D.) is inserted. A central venous pressure catheter is introduced percutaneously through an external jugular vein, and a femoral arterial catheter for monitoring is placed through a cut-down. Homologous heparinized sheep blood, stored overnight at 4°C is used to prime the bypass circuit and replace any blood loss. Potassium chloride, fluid to replace gastric drainage, and whole blood were given when indicated. Room temperature was kept at 30°C to minimize animal heat loss. Pulmonary ventilation was continued with 5 to 10 cm $H_2O$ continuous positive airway pressure. Heparin, 350 units/Kg, was given intravenously initially and in hourly doses of 150 units/Kg.

### *Human Perfusion*

Patients with acute severe respiratory failure who have deteriorated despite maximal respiratory and medical therapy are accepted for extracorporeal perfusion only after a hospital committee of independent disinterested physicians has reviewed their illness and confirmed their critical terminal condition. Using sterile surgical techniques, a venous drainage catheter (Table I) is inserted in the femoral vein under morphine analgesia (1 mg/Kg). Locating the tip of this drainage cannula radiographically at the level of the diaphragm insures minimal extracorporeal blood recirculation and adequate blood flow. In addition, a small cannula is placed in the distal femoral vein to provide venous drainage of the leg. The blood return cannula is placed in the internal jugular vein for venovenous perfusion, or in the femoral artery for venoarterial perfusion.

Patients 1 to 4 received an initial dose of 300 units/Kg heparin and then 100 units/Kg/hr by continuous infusion thereafter. Patient number 5 was given 20 to 40 units/Kg/hr maintenance doses of heparin to keep the Lee-White clotting time between 20 and 40 minutes.

Clinical, medical and respiratory management varied according to the clinical indications in each patient. All patients received antibiotics.

## Results

### *Characteristics of the Spinal Coil Membrane Lung*

Pressure drop across a 5 $M^2$ (28 cm wide) spiral coil lung is illustrated in Figure 5. It was 50 torr at 2.4 L/min and 110 torr at 6.4 L/min. The gas transfers across a recent Becton-Dickinson 5 $M^2$ lung are shown in Figure 6. Oxygen transfer was 50 ml/$M^2$/min at 5 L/min blood flow and an inlet saturation of 60 to 65%. Carbon dioxide transfer was 35 ml/min with a gas flow of 6.4 L/min and a venous $P_{CO_2}$ of 55 torr.

### *Total Cardiopulmonary Bypass in Adult Sheep*

Total respiratory gas exchange in adult sheep was achieved with large surface area (2.5 to 5 $M^2$) spiral coil membrane lungs. Total cardiopulmonary bypass for periods of up to 8 hours demonstrated the adequacy of blood flow and respiratory gas transfers in a simple system appropriate for clinical application. Gravity drainage of blood from 1 to 1.4 M provided flows up to 6.8 L/min (Table I). Oxygen flows through the lungs were 4.9 L/min. No thrombus was observed in membrane lungs on disassembly after termination of perfusion.

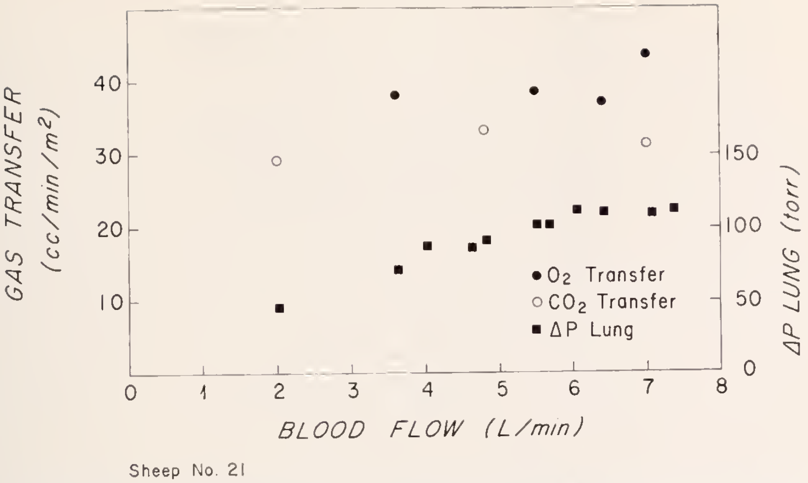

FIG. 5. Differential lung pressure and gas transfers for a 5 M$^2$ spiral coil membrane lung during veno-venous perfusion (Hg, 10 gms; Venous pH, 7.42; $P_{O_2}$, 44 torr; $P_{CO_2}$, 45 torr).

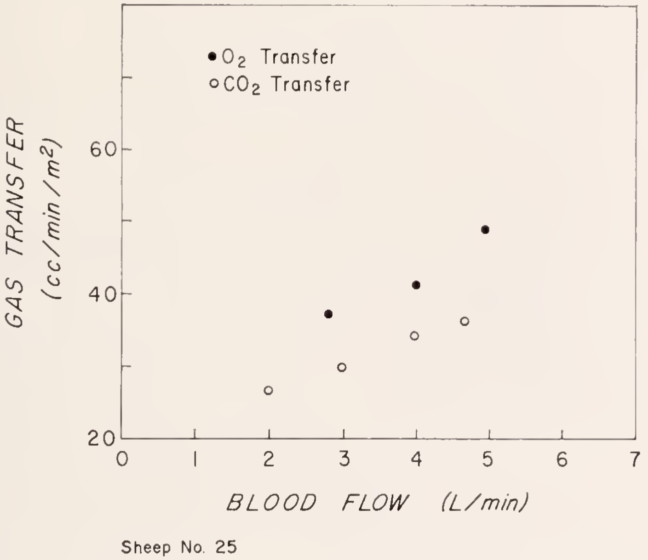

FIG. 6. Respiratory gas transfers for a 5 M$^2$ spiral coil membrane lung (Hg, 10 gms; venous pH, 7.36; $P_{O_2}$, 48 torr; $P_{CO_2}$, 55 torr).

No air emboli occurred in spite of significant membrane blood leaks during perfusions 1, 2, and 4.

Perfusion data from cardiopulmonary bypass in ten sheep for periods of one to eight hours are summarized in Table III. Average extracorporeal blood flow was 90 ml/Kg min and average $O_2$ consumption was 2.5 ml/Kg min. In early perfusions, there were multiple blood leaks due to the poor quality of the membrane. (Recent lungs both from Sci-Med and Becton-Dickinson have been

TABLE III

*Cardiopulmonary Bypass in Adult Sheep*

| Number | Kg | Hours | Area $M^2$ × No. | Peak Flow L/min | Hct % | $Pa_{O_2}$ Torr | $O_2$ Delivery ml/min | $PaCO_2$ Torr | Lowest Mixed $PvO_2$ Torr | Platelets $mm^3 \times 10^{-3}$ Initial-Final | Final Plasma Hgb mg% |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 49 | 6.5 | 2.5 × 2 (P) | 3.6 | 24 | 52 to 167 | 115 | 41 to 52 | 33 | 188–312 | 140† |
| 2 | 63 | 1 | 2.5 × 2 (S) | 4.7 | 30 | 50 to 108 | 205 | 44 to 58 | 33 | 386–232 | 4.4 |
| 3 | 45 | 4 | 2.5 × 2 (P) | 6.8 | 30 | 67 to 197 | 125 | 27 to 37 | 41 | 427–162 | — |
| 4 | 50 | 4 | 5.0 × 1 | 3.7 | 28 | 59 to 83 | 144 | 44 to 62 | 43* | 422–181 | 31 |
| 5 | 36 | 8.25 | 2.5 × 2 (P) | 3.7 | 34 | 59 to 110 | 78 | 30 to 37 | 37 | 97–110 | 28 |
| 6 | 47 | 8 | 2.5 × 2 (P) | 3.0 | 30 | 64 to 153 | 78 | 26 to 31 | 38 | 298–184 | 50 |
| 7 | 53 | 2.75 | 2.5 × 2 (S) | 4.5 | 28 | 75 to 350 | 130 | 25 to 48 | 63 | 469–201 | 104 |
| 8 | 54 | 1.75 | 2.5 × 2 (S) | 6.0 | 33 | 348 to 400 | 144 | 26 to 34 | 82 | 261–168 | 64 |
| 9 | 55 | 4.75 | 2.5 × 2 (S) | 4.7 | 28 | 239 tc 400 | 140 | 28 to 48 | 50 | 284–195 | 28 |
| 10 | 88 | 7.5 | 5.0 × 2 (P)‡ | 6.5 | 30 | 75 to 255 | 205 | 19 to 33 | 47 | 381–198 | 44 |

(S) = Series Circuit; (P) = Parallel Circuit.

* Leaking membrane lung.

† Prime blood, 1 week old.

‡ Prime, Ringer's lactate.

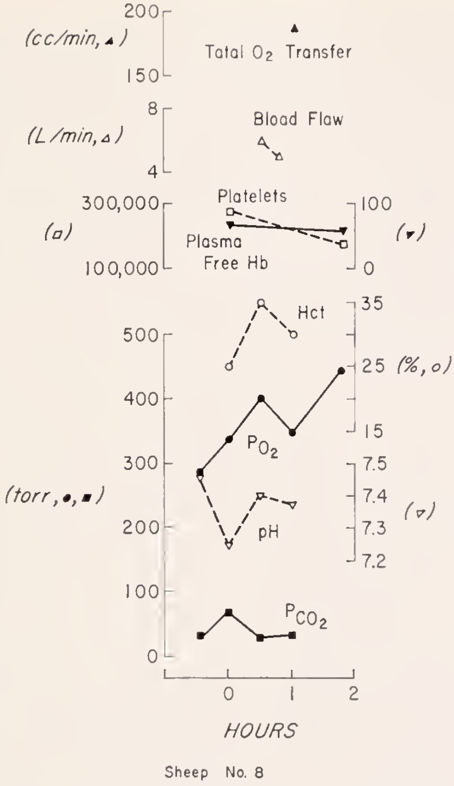

FIG. 7. Data obtained during cardiopulmonary bypass (54 Kg sheep; two 2.5 $M^2$ spiral coil membrane lungs in series).

pinhole free and have not leaked.) Because of the leaks the arterial $Po_2$ varied from 50 to 400 torr. The lowest mixed venous $Po_2$ was 33 torr, approximately 65% saturation. The pH and $Pco_2$ remained within a normal range without buffer administration. Platelet counts remained above 110,000/$mm^2$. Plasma free hemoglobin elevation increased with homologous blood transfusions and was not correlated with the length of perfusion.

Physiologic data collected during cardiopulmonary bypass in two sheep are illustrated in Figures 7 and 8. Animal number 8 had an arterial $Po_2$ greater than 300 with two 2.5 $M^2$ membrane lungs in series. Animal number 10, an 88 Kg sheep, was perfused with two parallel 2.5 $M^2$ membrane lungs for seven and one-half hours. Bypass was partial, and venous shunting accounts for the oxygen tensions less than 100 torr. On total bypass, the arterial $Po_2$ was 150 torr at one hour and 175 torr at seven and one-half hours. At a blood flow of 6 L/min, $O_2$ transfer was 205 ml/min, and $CO_2$ transfer was 201 ml/min.

The esophageal temperatures decreased to 32 to 33°C without a heat exchanger. With the heat exchanger, the temperature was maintained at 38°C.

Microscopic examination of the lungs showed periarterial and alveolar hem-

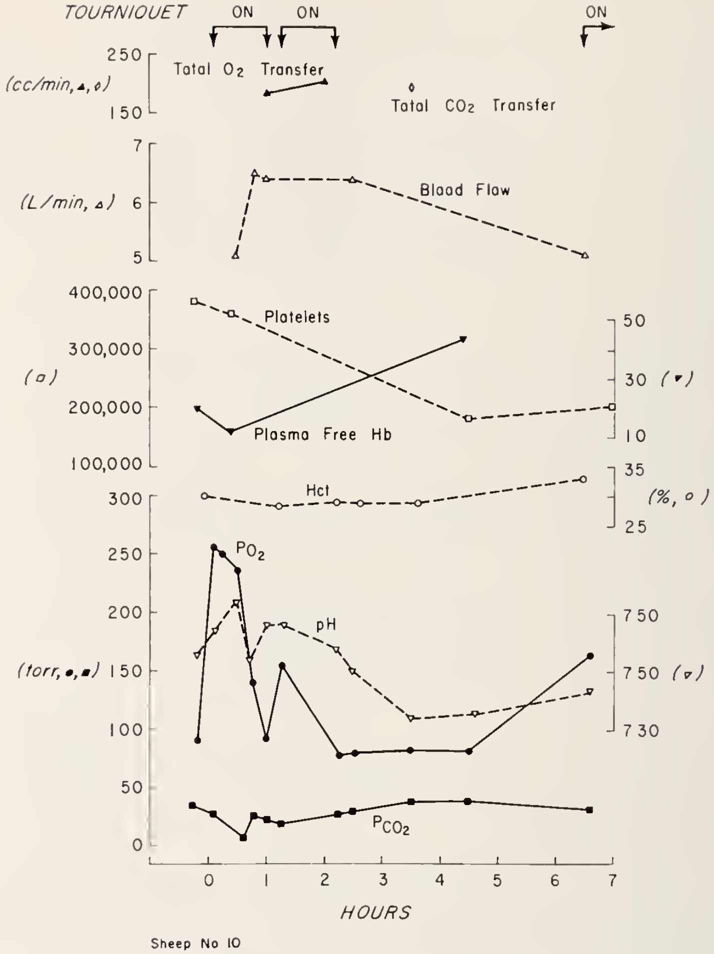

FIG. 8. Data obtained during cardiopulmonary bypass (88 Kg sheep; two 5 $M^2$ spiral coil membrane lungs in parallel).

orrhage. Microscopic examination of the lung was normal in an unheparinized ventilated control but showed alveolar (but not periarterial) hemorrhages in a heparinized and ventilated control.

### *Human Perfusion for Acute Respiratory Failure*

Clinical data and routes of perfusion are given in Table IV and cardiopulmonary data in Table V. Patient number 1 was perfused 60 hours. Her arterial oxygen tension, measured without extracorporeal assistance, improved during the period of extracorporeal gas exchange from 37 ($F_{IO_2}$ 1.0) to 50 torr ($F_{IO_2}$ 0.7). Ten days after perfusion was terminated, severe respiratory failure recurred and she died.

Patient number 2 died of circulatory collapse after 17 hours of adequate venovenous extracorporeal gas exchange.

TABLE IV

*Human Clinical Data and Perfusion Method*

| Patient | Age | Primary Disease | Perfusion Route | Time (Hrs) | Pulmonary Disease |
|---|---|---|---|---|---|
| 1 | 16 | Chronic Myelogenous Leukemia | VV | 60 | Pneumocystis Carinii Pneumonia |
| 2 | 17 | Peritoneal Stab Wound | VV | 17 | Septic Lung |
| 3 | 35 | Multiple Trauma | VA | 8 | Bronchopneumonia |
| 4 | 6 | Burns, ? Smoke Inhalation | VV | 43 | Bronchopneumonia |
| 5 | 29 | Barbiturate Intoxication, DIC | VV | 204 | Pulmonary Fibrosis & Hemorrhage |

VV = Venovenous; VA = Venoarterial.

TABLE V

*Cardiopulmonary Data during Perfusion*

| Patient | Weight (Kg) | Cardiac Output L/min | Bypass Flow L/min | Transpulmonary $O_2$ Transfer (ml/min) | Membrane Lung $O_2$ Transfer (ml/min/$M^2$) | Total Membrane Lung $O_2$ Transfer (ml/min) |
|---|---|---|---|---|---|---|
| 1 | 46 | — | 2.0 | — | 40.0 | 100 |
| 2 | 60 | 2.4 | 2.0 | 30 | 26.0 | 130 |
| 3 | 70 | — | 2.3 | — | 32.8 | 164 |
| 4 | 25 | 3.8 | 1.3 | 80 | 33.6 | 168 |
| 5 | 47 | 7.7 | 5.5 | 100 | 47.5 | 356 |

Venoarterial bypass was initiated after cardiac arrest in patient number 3 who had sustained multiple thoracic abdominal and long bone injuries. It was hoped to provide cardiopulmonary support for renal donation. There were hematoma and edema of the kidneys due to the initial injury and the kidneys were not used.

Patient number 4 was a six-year-old boy who suffered a 37 percent third degree body surface burn. His neck was severely burned, so axillary vein blood return was utilized. A second pump was required to return the oxygenated blood through the smaller bore cannula.

The patient was heparinized with 150 units/Kg/hr. No bleeding occurred from the eschar but massive blood replacement (20 units) was necessary due to blood loss from the groin cannulation site. He died in circulatory failure following severe intratracheal and pericardial hemorrhage.

Patient number 5 was a 29-year-old woman who was perfused 14 days after an overdose of barbiturates using three parallel 2.5 $M^2$ membrane lungs (Fig. 9). She had septicemia, peripheral gangrene, disseminated intravascular coagulation, and respiratory failure. Her $Po_2$ was falling and less than 50 torr while ventilated on 100 percent oxygen with 15 to 20 cm of positive end-expiratory pressure prior to venovenous perfusion. During perfusion, arterial $Po_2$ was kept at viable levels with a decreased $Fio_2$ and positive end-expiratory pressure. Extracorporeal blood flows over 6 L/min were obtained. At blood flows of 5.5

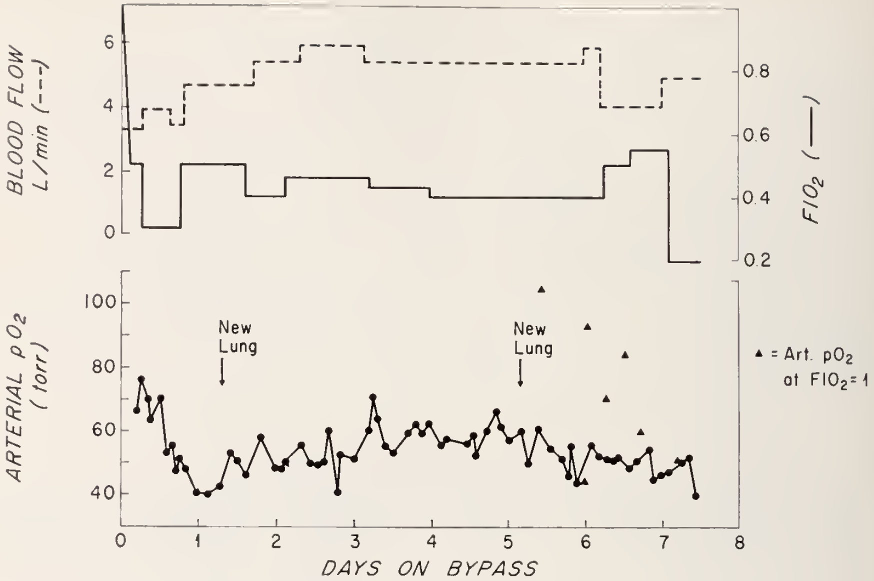

FIG. 9. Perfusion data from patient number 5. Reproduced with permission from Trans Am Soc Artif Int Organs 18: 553, 1972.

L/min, the membrane lungs provided 358 ml $O_2$ per minute, while the patient's lungs supplied 100 ml/min. The perfusion was terminated on the eighth day, after pulmonary bleeding rendered the patient unable to perform any gas exchange on her own, and she required total extracorporeal respiratory support. At autopsy, pathologic examination revealed extensive pulmonary fibrosis.

After an initial dose of 300 units/Kg, heparin doses in patient number 5 were lowered to 20 to 40 units/Kg/hr to keep the Lee-White clotting time between 20 and 40 minutes. Despite distal drainage of the lower limb, electrocautery, and short clotting times, the patient developed massive bleeding from the groin cannulation site requiring multiple transfusions. Platelet counts which had been maintained above 200,000/mm$^3$ during the first 24 hours of perfusion, fell to below 50,000/mm$^3$. The heparin infusion was discontinued. Clotting times stabilized at ten minutes and bleeding ceased. At the end of 36 hours without anticoagulation, the platelet count rose to over 50,000/mm$^3$ and heparin was reinfused at 20 units/Kg/hr to prevent coagulation and thrombosis within the membrane lungs. Thereafter, the Lee-White clotting time was maintained at 15 to 20 min. One membrane lung developed thrombi and was replaced. One membrane lung had been replaced after 24 hours because a small leak from blood to gas phase had occurred.

In three of the five patients, plasma-free hemoglobin remained under 15 mg percent. In four patients heparinization was initially 300 units/Kg and maintained with 100 units/Kg/hr by continuous infusion with Lee-White clotting times over four hours. Patient temperatures were stable at 35 to 37°C without a heat exchanger.

## Discussion

This article describes a simple system using disposable spiral coil membrane lungs for cardiopulmonary bypass and prolonged respiratory support in adults. Adequate blood flow was obtained with gravity drainage of venous blood through the membrane lungs. Thin-walled cannulae were used to assure maximum blood flow. Gravity drainage through the membrane lungs minimizes the transmembrane pressure; pumping the blood through the oxygenators applies greater pressures across fragile membranes. Lower pressures across the membranes decrease the possibility of blood leaking into the gas phase through a pinhole. Such leaks can decrease the efficiency of gas transfer.

Adequate surface area must be used for total gas exchange. Blood flows of 0.5 to 1 L/$M^2$ and membrane surface area of 1.0 $M^2$/10 Kg allow a minimum of 4 ml $O_2$/Kg/min of extracorporeal oxygen and carbon dioxide transfer and thus permits total respiratory gas exchange. It may be necessary to be able to perform total patient carbon dioxide exchange as well as oxygen exchange. The spiral coil membrane lung has this capacity. There is rarely an excuse for inadequate extracorporeal gas exchange; the risks of extracorporeal perfusion are incurred regardless of the membrane surface area used. Surface area can be increased with a minimal increase in priming volume (Table II).

Minimal blood damage occurs during prolonged perfusion with careful attention to the materials contacting blood, flow dynamics, and nonocclusive pumping. During cardiopulmonary bypass, significant blood damage was not incurred, however, we did not use cardiotomy suction return.

Although no heat exchanger was used for long-term clinical perfusion, temperatures were kept stable at 35 to 37°C by enclosing the apparatus in a vinyl canopy maintained at 40°C.

Acute loss of blood volume decreases venous return. No shift of volume from the patient can occur with a fixed-volume extracorporeal perfusion circuit. For clinical cardiopulmonary bypass, a regulated blood reservoir will be necessary to allow rapid replacement of operative blood loss.

When consistently reliable silicone rubber membrane is available at more reasonable cost to the patient, membrane oxygenators will provide simple, and safer techniques for cardiopulmonary bypass. Current costs (over \$90/$M^2$) severely limit the widespread application of these devices to cardiac surgery.

The essential factors in long-term perfusions are: 1) meticulous attention to blood surface contact; only silicone rubber, tetrafluoroethylene, or segmented polyurethane surfaces contact blood, 2) minimal blood suction; only 0.7 to 1.0 meter hydrostatic blood drainage is allowed; and 3) pumping with a non-occlusive large bore chamber. A fiberglass reinforced polyurethane pump chamber is used to eliminate the possibility of rupture caused by prolonged occlusive pumping with vinyl or silicone rubber tubing.

In addition, the enlarged and modified spring steel reinforced segmented polyurethane venous drainage cannulae with side holes allow the large stable blood flows necessary for adequate extracorporeal gas transfer in adults. In patient number 5, cannulated with a 9.5 mm I.D. catheter, we achieved blood flows greater than 8 L/min without "tubing flutter." When the catheter was properly placed, there was minimum recirculation of blood. Despite its large size, the catheter was readily placed in the femoral vein of this small patient. We have constructed a 12.7 mm I.D. catheter for even larger patients.

The pulmonary disease under consideration must be deemed reversible. A decision to institute extracorporeal support must be based on the patient's clinical course as well as his arterial gas tensions under maximal respiratory and medical management. The risk of continuing high positive end-expiratory pressure and prolonged high inspired $O_2$ concentrations must be balanced against the risks of cannulation, heparinization, and prolonged extracorporeal perfusion. Bleeding at surgical and trauma sites is a major concern. Venoarterial perfusion should be considered if cardiac failure is a contributing factor. The advantages or disadvantages of venovenous and venoarterial perfusion were recently reviewed by Zapol (8).

Prolonged extracorporeal respiratory support is now possible for patients with acute reversible respiratory failure. Our experience suggests that an early decision to begin extracorporeal support is essential to successful perfusion. Continued experience in the care of these critically ill patients is necessary to define the role of this new technique in their management.

## Summary

To demonstrate the adequacy of blood flow and gas transfer of the spiral coil membrane lung, ten adult sheep (35 to 88 Kg) were placed on cardiopulmonary bypass for periods up to 8 hours. A simple system using large surface area 2.5 $M^2$ and 5 $M^2$ spiral coil membrane lungs allowed gravity drained blood flows up to 6 L/min through two parallel lungs. Oxygen delivery of up to 205 ml/min was obtained. Arterial $Po_2$ ranged from 50 to 400 torr, and $Pco_2$ from 25 to 58 torr. Platelet counts always remained above 100,000/$mm^3$. The spiral coil membrane lung provides adequate gas transfer in this simple system appropriate for clinical use.

Clinical experience with prolonged extracorporeal perfusion for terminal acute respiratory failure is reviewed. Venovenous perfusion was used in four patients and venoarterial perfusion in one. There were no long-term survivors, although one patient was improved after 60 hours and lived ten days after perfusion was discontinued. Adequate respiratory support with an arterial $Po_2$ over 60 torr was maintained for eight days in one patient. A major problem was bleeding from tracheal, retroperitoneal space and cannulation sites even at heparin doses of only 20 to 30 units/Kg/hr.

### Acknowledgments

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# Clinical Use of the Membrane Lung Oxygenator for Acute Respiratory Insufficiency

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In the past few years, improvements in the rapid clinical assessment of respiratory mechanics in postoperative and post-traumatic patients have led to earlier and better management in those individuals who may subsequently develop acute respiratory insufficiency. Nevertheless, a variety of complications can occur when mechanical ventilation and high oxygen concentrations are necessary for prolonged respiratory support in the critically ill patient. The recent development of disposable membrane oxygenators appears to be a valuable tool for long-term artificial oxygenation in potentially reversible pulmonary insufficiency. The present report outlines the authors' experiences with the use of venovenous extracorporeal perfusion as a treatment for acute respiratory failure.

## Case Material and Technique

Five patients with severe respiratory insufficiency were evaluated for continuous membrane lung support. One had a cardiac arrest in the operating room and expired prior to insertion of the bypass cannula while the remaining four underwent extracorporeal oxygenation which varied from 6 hours to 5 days (Table I). Indications for initiating this form of therapy in hypoxemic patients were: 1) $Pa_{O_2}$ less than 35 mm Hg despite an $F_{IO_2}$ of 100 percent on a volume-controlled respirator; 2) Potentially reversible pulmonary failure; and 3) Hypoxemic central nervous system or cardiovascular deterioration.

A veno-venous bypass technique using the General Electric-Peirce membrane lung was employed in all four patients. Under local anesthesia a wire reinforced vinyl cannula was inserted at the sapheno-femoral vein junction and positioned well up into the inferior vena cava. Through a separate neck incision a second cannula was placed in the right atrium via the internal jugular vein. Careful attention was paid to sterile technique and meticulous hemostasis. Each patient received 300 units/Kg of heparin prior to cannulation. The extracorporeal circuit was primed with fresh pretyped whole blood. Blood priming volume and oxygen supply flow rates varied with the size of the membrane lung employed. Blood was supplied via the caval catheter to a venous reservoir and then pumped into the lung. After oxygenation the blood was pumped through a bubble trap and back to the right atrium. Body temperature was held to 35 to 37°C. by a heat exchanger in the oxygenator. As soon as possible after initiation of bypass, patients were ventilated with 40 to 60 percent oxygen. Manometers

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TABLE I

*Case Material—Venovenous Bypass*

| Patient | Age | Underlying disease | Pulmonary lesion | Perfusion time |
|---|---|---|---|---|
| H.W. | 38 | Chronic kidney failure | Bacterial pneumonia | 6 hours |
| V.B. | 66 | Tuberculosis | Bacterial pneumonia | 8 hours |
| G.W. | 11 | Acute lymphocytic leukemia | P. carinii pneumonia | 4.5 days |
| C.R. | 36 | Renal transplant | P. carinii pneumonia | 5 days |

recorded inlet and outlet pressures in the circuit. A second standby lung was mounted in parallel in case of leakage or for use should the patient's oxygen demand require it. A flow meter on the heart-lung machine monitored bypass flow rates. All patients were kept in the Special Care Unit where catheters were inserted for continuous monitoring of central venous pressure and arterial pressure. Arterial blood gases, hemoglobin, and clotting studies were performed at intervals varying with the patients condition. All data were recorded on an Alpha 21 analog and digital computer. Maintenance heparin dosage was 100 units/Kg/hr in case 1 and 2.

*Case 1.* H.W., a 38-year-old housewife, had been on chronic dialysis for the last 5 years due to complete kidney failure secondary to streptococcal glomerulonephritis. She was admitted to the hospital for "flu-like" symptoms consisting of fever, chills, chronic malaise, and diarrhea. Initial laboratory studies were unremarkable except for a slight leukocytosis. Admission chest roentgenograms showed diffuse bilateral pulmonary infiltrates. During the next 72 hours pulmonary consolidation progressed and the patient became hypoxemic with a $Pa_{O_2}$ of 65 mm Hg on room air. Tracheostomy and volume controlled ventilator assist were instituted because of continued deterioration. The $Pa_{O_2}$ fell to 31 mm Hg on 100% oxygen ($o_2$ Sat. 52%) and right heart failure developed necessitating digitilization. Because of a lack of pulmonary improvement on 100% $O_2$ and despite a falling systolic blood pressure (50–60 mm Hg) she was placed on venovenous bypass utilizing a G.E. 2 $M^2$ lung. During the six-hour perfusion venous blood entering the membrane had the following mean values: $Pa_{O_2}$ 39 mm Hg, $Pa_{CO_2}$ 60 mm Hg, $o_2$ Sat. 66%. Arterial blood returning to the patient had mean values of $Pa_{O_2}$ 349 mm Hg, $Pa_{CO_2}$ 29 mm Hg, $O_2$ Sat. 99%. Although initial pump flow was 1.5 L/min, this gradually began to fall to less than 500 ml/min due to the patient's continued poor cardiac output and systemic hypotension. She expired 2 hours after bypass was discontinued.

*Comment:* In retrospect, because of poor cardiac output, this patient was not an ideal candidate for venovenous bypass since adequate inflow into the membrane lung could not be maintained. Possibly venoarterial bypass or venovenous perfusion with intraaortic balloon pump assist would have been more appropriate. Blood gas studies in this patient do demonstrate effective oxygenation across the lung but at flow rates inadequate to sustain the patient.

*Case 2.* V.B., a 66-year-old woman known to be a heavy smoker with emphysema and a tuberculosis cavity in the right lung, was admitted because of shortness of breath, cough and fever. Initial chest roentgenograms showed a right upper lobe cavity as well as diffuse bilateral pulmonary infiltrates. Cultures obtained at bronchoscopy grew out acid-fast organisms and diplococci. She was placed on appropriate antibiotics and antituberculous medications.

Admission blood gas studies were adequate ($Pa_{O_2}$ 84 mm Hg). Over the next two weeks her pulmonary infiltrates progressed to extensive bilateral consolidation, and her $Pa_{O_2}$ varied between 38–47 mm Hg on 100% oxygen with a volume-controlled ventilator. Because of the continued need for high oxygen concentrations, she was placed on venovenous bypass utilizing a 2 $M^2$ G.E. membrane lung. While on the lung, flow rates averaged 2 L/min. Blood entering the circuit showed the following mean values: $Pa_{O_2}$ 46 mm Hg, $O_2$ Sat. 66%. Oxygenated blood leaving the membrane lung had the following mean values: $Pa_{O_2}$ 86 mm Hg, $O_2$ Sat. 99%. Despite adequate membrane oxygenation the patient's arterial blood gas values were only $Pa_{O_2}$ 39–40 mm Hg. Because of congestive heart failure and evidence of irreversible cerebral deterioration, perfusion was ended after 8 hours and she expired.

*Comment:* This patient also had heart failure and possibly would have benefitted from venoarterial bypass. The fact that she had a low $Pa_{O_2}$ on a ventilator despite 100 percent oxygen for ten days with obvious central nervous and cardiac deterioration suggest that candidates for bypass should be seen earlier before the detrimental effects of high concentrations of inspired oxygen and hypoxemic cerebral death have occurred.

*Case 3.* G.W. was an 11-year-old boy under treatment for acute lymphocytic leukemia with steroids and methotrexate. He developed fever, cough with progressive pulmonary failure and a roentgenographic picture of bilateral hilar infiltrates. A needle biopsy of the lung on the second hospital day confirmed a diagnosis of Pneumocystis carinii pneumonia. Treatment was instituted with pentamidine 4 mg/Kg/day. Because of continued pulmonary parenchymal consolidation and increasing hypoxia, tracheostomy was performed and respirator support instituted. Forty-eight hours later despite an $Fi_{O_2}$ of 100%, arterial blood gas studies revealed a $Pa_{O_2}$ of 37 mm Hg, $Pa_{CO_2}$ 42 mm Hg and pH 7.45. He was begun on venovenous bypass utilizing a 1 $M^2$ G.E. membrane lung with a flow of 750–900 ml/min. During the next 4½ days the patient's mean arterial blood gas studies showed a $Pa_{O_2}$ 57 mm Hg, $Pa_{CO_2}$ 38 mm Hg, $O_2$ Sat. 94% with the ventilator delivering an inspired oxygen concentration of 50%. With a maintenance heparin dose of 150 units/Kg/hr and a partial thromboplastin time (PTT) greater than 300 sec, 106 hours after bypass was begun, he developed gastrointestinal bleeding with the passage of bright red blood and tarry stools and gross hematuria. Four hours later bypass was discontinued and the heparin was reversed with protamine sulfate. Initially following bypass he could be managed on the ventilator with a $Pa_{O_2}$ of 58 mm Hg on 80% oxygen. However, by the following morning he gradually became more hypoxemic, developed grand mal seizures, and finally had a cardiac arrest and could not be resuscitated.

*Comment:* This young man was an excellent candidate for membrane lung assist. He had a potentially reversible pulmonary lesion and bypass was begun early. Indeed while on the lung his pulmonary consolidation gradually improved. He did experience some benefit from extracorporeal oxygenation in that following bypass his $Pa_{O_2}$ was 58 mm Hg on 80 percent oxygen which we were unable to attain prior to membrane lung assist. The problem here was poor heparin control since a PTT of 200 appears to be adequate while patients are on the artificial lung.

*Case 4.* C.R., a 36-year-old man, had undergone renal allografting 7 months prior to admission and current immunosuppressive therapy included steroids, azathioprine, and antilymphocyte globulin. At the time of admission he complained of fever, malaise, nonproductive cough and shortness of breath. Arterial $Pa_{O_2}$ was maintained at 76 mm Hg on room air. During the following four days the patient developed progressively increasing dyspnea and chest roentgenograms showed diffuse pulmonary infiltrates with bilateral hilar consolidation. Needle biopsy confirmed the diagnosis of Pneumocystis carinii pneumonia and pentamidine 4 mg/Kg/day was begun. $Pa_{O_2}$ fell to 31 mm Hg despite an $F_{IO_2}$ of 100%. Veno-venous bypass was instituted utilizing a 2 $M^2$ G.E. membrane lung with a flow of 2.3 L/min. During the next 4½ days the patient's arterial $Pa_{O_2}$ varied between 46–54 mm Hg while the inspired oxygen concentration through the ventilator was reduced to 60%. After 101 hours of bypass with a $Pa_{O_2}$ of 54 mm Hg, bypass flow was progressively decreased. The patient continued to maintain adequate oxygenation, and bypass was stopped 12 hours later. With ventilator assistance ($F_{IO_2}$ 60%), arterial $Pa_{O_2}$ continued to rise (55 to 85 mm Hg). After 10 days of pentamidine therapy, the pulmonary infiltrates gradually resolved and he was able to sustain an arterial $Pa_{O_2}$ of 85 to 90 mm Hg on room air. The patient subsequently made an uneventful recovery and was discharged.

*Comment:* As with the last case, P. carinii pneumonia represents a potentially reversible pulmonary lesion. In addition, this patient was kept on a high oxygen concentration (100%) for only 48 hours before membrane oxygenation was instituted. During bypass, extracellular fluid retention did not appear significant. On two occasions when urine flow diminished, intravenous furosemide produced a prompt diuresis. Four units of packed red cells were given during the course of bypass because of a falling hematocrit.

## Discussion

Although basic blood oxygenators have been utilized for many years, it was not until Clowes (1) demonstrated the feasibility of a blood-gas exchange based on a membrane separation that prolonged extracorporeal oxygenation appeared practical. Later Peirce (2) showed that the thickness of the blood boundary layer was an important consideration in the gas-transfer capacity of the membrane lung. The advent of silicone copolymers has now provided membranes with adequate structural strength and reliable gas diffusion properties (3). Excellent long-term perfusion can now be performed with a relative lack of toxic effects to the blood elements. Currently a number of clinical and research studies utilizing a portable disposable membrane oxygenator have been undertaken by Peirce, Landé, Kolobow, Hill and others with gratifying results (4–7). Venovenous bypass is theoretically more promising in potentially reversible acute respiratory insufficiency. It can be rapidly initiated and appears to alter cardiac hemodynamics only slightly. In addition, satisfactory arterial oxyhemoglobin levels can be readily attained with a low bypass flow. Hill, Haller, and others (8, 9) have reported satisfactory pulmonary support clinically with this method.

In the present series of patients treated by venovenous bypass, blood gas studies demonstrated more than adequate oxygenation across the membrane ($O_2$ Sat. 99%). The first two cases were unsuitable candidates. The first patient had a low cardiac output and so adequate flows to the circuit were not obtained, an important pre-requisite in venovenous bypass. Perhaps intraaortic balloon pump assist should have been established as demonstrated by Lefemine (10). In the second individual, oxygen toxicity and hypoxemic cerebral death had already occurred. This points up the need for more clinical experience allowing earlier institution of membrane lung support.

The last two patients undergoing bypass for four and one-half and five days were good candidates for extracorporeal oxygenation. A potentially reversible pulmonary lesion was present and therapy was instituted early. Both had a $\mathrm{Pa}_{\mathrm{O}_2} < 40$ mm Hg despite an $\mathrm{F}_{\mathrm{IO}_2}$ of 100%. The flow rates obtained represented 40–45% of the cardiac output and attained a $\mathrm{Pa}_{\mathrm{O}_2}$ of 45–57 mm Hg with a ventilator delivering an inspired oxygen concentration of 60 percent. This improved oxygenation obviously was sufficient to maintain these patients, while the lungs environment was more suitable for recovery. Both of these individuals did show transient leukocytosis and thrombocytopenia as reported by others (4, 8). The complications of prolonged anticoagulation in Case 3 were overcome in Case 4. We now utilize a constant dilute heparin infusion (15–20 units/Kg/hr) which maintains a Lee-White clotting time of 25 to 45 min—a protocol suggested by Hill's extensive experience (11). Case 4 shows the rapid improvement in lung function that occurs following perfusion. At four and one-half days we were able slowly to wean the patient off bypass maintaining an adequate $\mathrm{Pa}_{\mathrm{O}_2}$ while on only 60 percent oxygen. Twelve hours later with no membrane lung support the $\mathrm{Pa}_{\mathrm{O}_2}$ was 55 mm Hg. Eventual decrease in the pulmonary shunt was demonstrated by a $\mathrm{Pa}_{\mathrm{O}_2}$ of 201 mm Hg when the $\mathrm{F}_{\mathrm{IO}_2}$ was temporarily increased to 100 percent.

## Summary

Current developments in membrane lung designs have now allowed long-term extracorporeal pulmonary support to be performed with a minimum of complications. Four cases are presented which demonstrate the clinical usefulness and indications for external oxygenation. Two individuals had long-term extracorporeal oxygenation for a reversible primary pulmonary lesion; both showed adequate arterial oxygen concentrations while on bypass. The last patient points out the beneficial effect of membrane lung support if instituted early.

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# The Use of Hyperbaric Oxygenation in Conjunction with Membrane Lung Perfusion for Respiratory Insufficiency

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Extracorporeal support with the membrane lung has been shown to be effective in treatment of severe acute respiratory insufficiency (1, 2). Since respiratory failure occurs in conjunction with low cardiac output as may be the case after open heart surgery, venovenous perfusion may not be very supportive because it does not improve systemic blood flow. Venoarterial perfusion may not be possible at a high enough flow to provide necessary gas exchange without unduly raising the left atrial and pulmonary capillary pressures. In such circumstances, oxygen transferred per unit volume of perfused blood can be at least doubled by carrying out the perfusion under hyperbaric conditions. A single clinical case is presented to show the effectiveness of the membrane lung combined with hyperbaria. A previous hyperbaric case was reported by Weinreich and Lipton (3).

### Case Report

The patient was a 35-year old white woman with severe biventricular failure due to rheumatic heart disease. Preoperatively she had a grade V to VI holosystolic murmur and a grade III to VI diastolic blow along the left sternal border, atrial fibrillation, cardiomegaly, hepatomegaly to four fingerbreadths, and water hammer peripheral pulses. A triple valve replacement was carried out on October 6, 1971, using a Björk-Shiley aortic, a Starr-Edwards mitral, and a porcine heterograft tricuspid valve. The patient was on total cardiopulmonary bypass, using a bubble oxygenator, for four hours and twenty-five minutes. While coming off the pump, and without unusual left atrial pressure elevation, there was a sudden episode of pulmonary edema. Subsequently there was difficulty in maintaining the arterial pressure and the arterial oxygen tension ($Pa_{O_2}$) despite the use of digitalis, diuretics, steroids, isuprel, epinephrine, and intermittent positive pressure ventilation on 100% oxygen at inflation pressures up to 70 cm $H_2O$. Positive end expiratory pressure (PEEP) (4) caused a precipitous decline in the arterial pressure and could not be used. The patient was poorly responsive and had evidence of right-sided weakness. Because of continuing pulmonary edema, a $Pa_{O_2}$ of 38 torr (mm Hg) on 100% $O_2$ and 70 cm $H_2O$ positive pressure, a left pneumothorax, low arterial pressure, and poor responsiveness, the patient was transferred to the hyperbaric chamber 24 hours postoperatively (Figs. 1 and 2).

On high oxygen tensions at pressures up to two atmospheres absolute (2 ATA), the pulmonary edema subsided, pulmonary compliance improved, $Pa_{O_2}$ rose, and carbon dioxide tension ($Pa_{CO_2}$) fell. PEEP was still not possible and hyperbaric oxygen could not be discontinued.

Two days postoperatively venoarterial perfusion was elected. This was started with the

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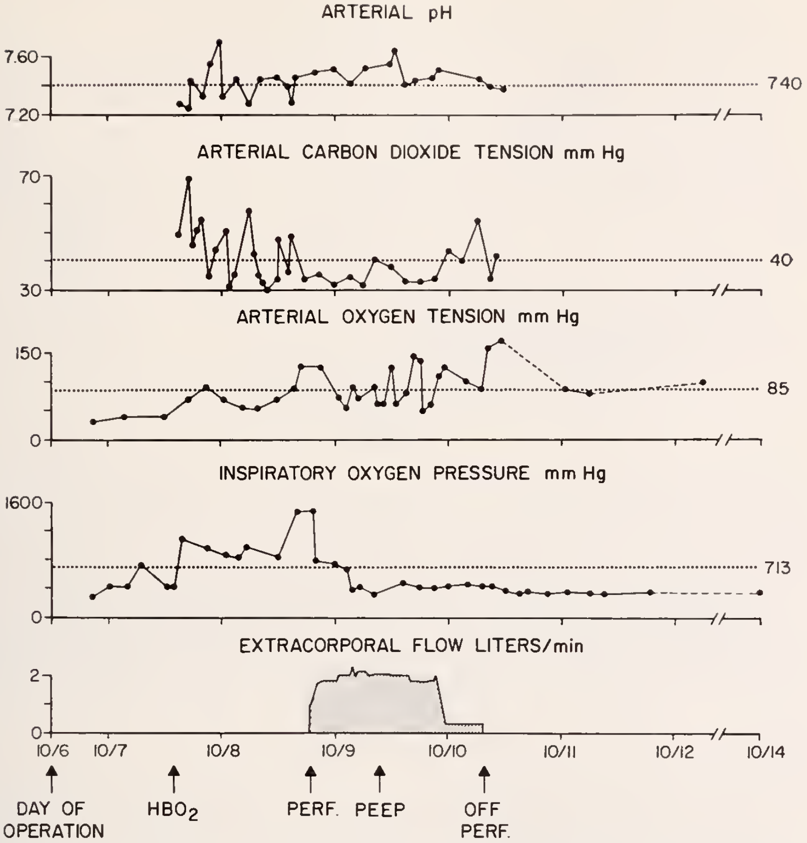

Fig. 1. (See also Figs. 2 and 3.) Prior to the start of hyperbaric oxygenation ($HBO_2$) arterial oxygen tension ($Pao_2$) remained between 30 and 50 mm Hg (torr) even when the inspiratory oxygen pressure was over 700 mm Hg ($Fio_2$ approximately 100%). The arterial pH was low and the arterial carbon dioxide tension ($Paco_2$) high because tidal volume was low despite a ventilatory pressure of 70 cm $H_2O$ (Figure 2). On $HBO_2$ compliance improved somewhat and it was possible to bring the $Pao_2$ up to a normal range and the $Paco_2$ down with inspiratory oxygen pressures between 800 and 1400 mm Hg. There was some improvement in tidal volume and minute volume at inspiratory pressures of about 50 cm $H_2O$ as pulmonary edema subsided (Figure 2). It was not possible, however, to discontinue the $HBO_2$. On perfusion, extracorporeal flows up to two liters per minute were possible, $Pao_2$ remained above 50 mm Hg, and pH and $Paco_2$ stabilized. Blood pressure (and probably cardiac output) improved (Fig. 3) and it was now possible, for the first time, to use positive end expiratory pressure (PEEP) which improved tidal volume and lowered inflation pressures (Fig. 2). Inspiratory oxygen pressure could be stabilized at the equivalent of an $Fio_2$ of 50 to 60%. $HBO_2$, and soon thereafter perfusion, could be discontinued. The patient's pulmonary situation continued to improve but a respirator and PEEP were necessary until brain death was diagnosed four days later (Fig. 2).

patient at 2 ATA on 100% oxygen to provide a high $Pao_2$ (Fig. 1). A 2 $M^2$ General Electric-Peirce membrane lung (5) was employed in conjunction with an Olson pump equipped with a weighing arm that controlled an automatic arterial pump. Because of a high left atrial pressure, the perfusion was started slowly. Immediate hemodynamic improvement was noted (Fig. 3). The patient gave up about one liter of blood to the circuit and the arterial pressure stabilized within normal limits. Perfusion flows up to two liters per

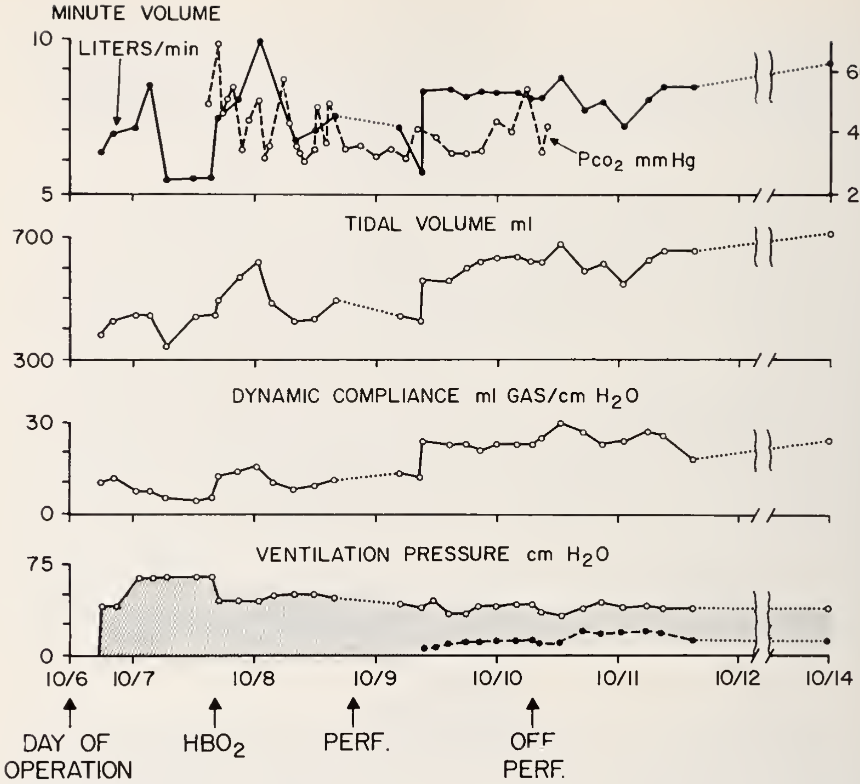

FIG. 2. (See also Figs. 1 and 3.) Some of the principal ventilatory features are illustrated. Note the very low dynamic compliance, low tidal and minute volumes, and the high Paco$_2$ postoperatively despite a ventilatory pressure of 70 cm $H_2O$. On $HBO_2$, the ventilatory pressure could be reduced to about 50 cm $H_2O$ as compliance and ventilation improved. The beneficial effect of the $HBO_2$ was limited to the early resolution of the pulmonary edema and $HBO_2$ had to be continued to maintain the Pao$_2$ (Figure 1). The most important effects of perfusion were moderate improvement in Pao$_2$ and Paco$_2$, and a striking hemodynamic effect (Fig. 3). PEEP led to an abrupt and marked increase in the tidal and minute volumes and in the dynamic compliance. The improvement in respiratory dynamics was progressive over the remaining four days.

minute were generally possible but the flow could not be increased without dangerously elevating the left atrial pressure. Extracorporeal oxygen exchange averaged about 100 ml per minute, about 50% of which consisted of dissolved oxygen (Fig. 4). The Pao$_2$ improved and it was possible to reduce the oxygen tension supplying the natural lung to 400 mm Hg (torr) or less, equivalent to an Fio$_2$ of about 60% without hyperbaria. This is an Fio$_2$ not likely to cause or increase oxygen toxicity. With the perfusion, it was possible to institute PEEP and this was accompanied by improvement in the pulmonary compliance (Figs. 1 and 2). It was then possible to discontinue the hyperbaric oxygenation and after 30 hours to start weaning the patient from the pump. Perfusion was discontinued after 36 hours.

A tracheostomy was required during the perfusion to permit removal of a nasotracheal tube and nasal packing for epistaxis. Additional blood loss occurred from the tracheostomy and from a myringotomy that was necessary because of the hyperbaria. No troublesome bleeding occurred from the bilateral chest tubes. The heparin level was followed with fair success by means of the whole blood activated partial thromboplastin time (BAPTT) (Fig. 6). Bleeding from the tracheostomy was controlled when the BAPTT stabilized at about 200 to 400 seconds (3 to 6 times normal) on only 20 units of heparin per kilogram per hour. The

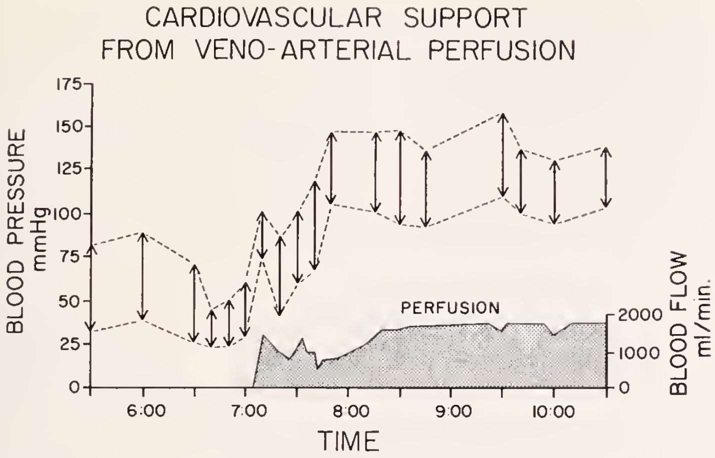

FIG. 3. Prior to perfusion, the patient had severe combined respiratory and cardiovascular failure. Respiratory changes are shown in Figs. 1 and 2. Despite $HBO_2$ at 2 ATA on 100% $O_2$, blood pressure ranged from about 50/25 to 90/40. As soon as perfusion was started, blood pressure returned to a normal range and about one liter of blood was given up to the circuit during the next 30 minutes. The left atrial pressure (LAP) was very sensitive to the extracorporeal flow and, for a time, flow had to be adjusted frequently to prevent a dangerously high LAP and recurrence of pulmonary edema. The early variations in flow are clearly shown in the figure. After about one hour, it was possible to maintain the flow at just under 2 L/min with an LAP not exceeding 15 to 18 cm $H_2O$. Figures 3 and 6 reproduced by permission of Chest (2).

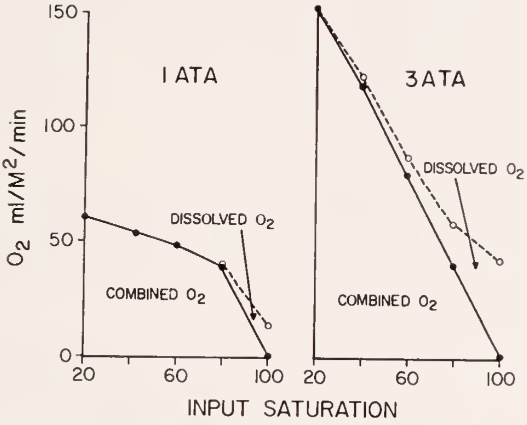

FIG. 4. The relationship of dissolved oxygen to oxygen combined with hemoglobin is illustrated for the General Electric-Peirce membrane lung at 1 ATA and 3 ATA of oxygen. The figure assumes a blood flow of 1 L/min./$M^2$ through the artificial lung and a normal hematocrit. At 1 ATA full saturation (95% or more) is reached for blood entering at 75% or higher saturation. Dissolved oxygen ($O_2$) is negligible until there is full saturation since it is only 0.3 vol % for each 100 mm Hg $Po_2$. The maximum dissolved $O_2$ is about 1.8 vol % or 18 ml/L blood flow/min. At 3 ATA, blood entering the lung will almost always reach full saturation and the dissolved $O_2$ may reach 45 ml/L/min or more. In the present case, 2 ATA was used and the lung outlet $Po_2$ values were measured at about 1100 mm Hg, indicating a dissolved $O_2$ level of 33 ml/L/min. As the blood flow was nearly 2 Liters/min, actual exchange as dissolved $O_2$ is estimated at 50 ml/min or more, equal to 50% or more of the entire membrane lung $O_2$ exchange.

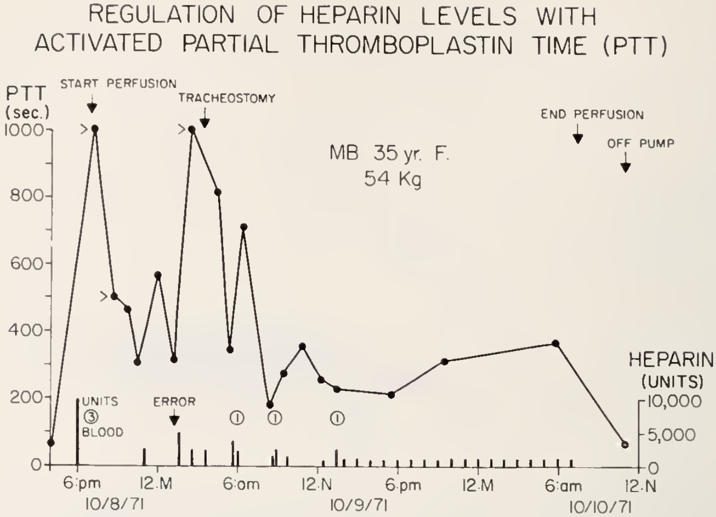

Fig. 5. Regulation of the heparin level by means of the whole blood activated partial thromboplastin time (BAPTT) is illustrated. The 50 Kg patient was given 200 units of heparin/Kg initially (10,000 u) plus 7500 u in the 3 units of whole heparinized blood used to prime the circuit. The BAPTT was over 1000 sec (control 65 sec) but gradually fell to 300 sec in the next 4 hours. An error report of immediate clotting, later shown correctly to be a BAPTT of 300 sec, prompted the administration of 5000 u of heparin and the BAPTT again exceeded 1000 sec. Soon thereafter a tracheostomy became necessary and was followed by substantial blood loss from oozing, requiring a transfusion of 3 units of heparinized blood. Only when the BAPTT approached 200 sec was this controlled. Heparin maintenance was eventually stabilized at 20 u/Kg/hr which kept the BAPTT between 200 and 400 sec. Almost certainly it would have been desirable to give somewhat less heparin and maintain the BAPTT at 2 to 3 times normal. After perfusion was discontinued, the BAPTT reached the preperfusion level within 4 hours without heparin neutralization. Reproduced by permission of Appleton-Century-Crofts, New York, *Surgery Annual 1972* Edited by P. Cooper and L. Nyhus (5).

plasma hemoglobin reached a maximum of 29 mg% and the platelet level was about 50,000/mm$^3$.

During and following the perfusion the patient showed progressive neurologic deterioration. She was maintained with satisfactory respiratory and renal function for five days. At this time a determination of cerebral death was made and she became a kidney donor. One kidney was unsatisfactory because of an accessory artery. The second kidney showed delayed function after transplantation but has performed well and the recipient now has satisfactory renal function after nine months.

Autopsy revealed intracranial hemorrhage thought to be secondary to embolic brain damage. The lungs were heavy and showed congestion, edema, focal hemorrhages, and bronchopneumonia. There were focal areas of intraalveolar organization with fibroblastic proliferation. Homogeneous acidophilic fibrinous material was scattered on the alveolar surfaces, and alveolar lining cells showed hyperplasia and atypism. Some hemorrhagic thrombi were present in small pulmonary arteries. The heart was moderately enlarged. The three implanted valves were well placed and free of significant thrombi. The coronary arteries were patent and without sclerotic change. The endocardium was thickened and there were subendocardial hemorrhages.

## Discussion

Modern intensive respiratory care has markedly improved the salvage rate in all types of acute respiratory insufficiency. Knowledgeable use of positive pres-

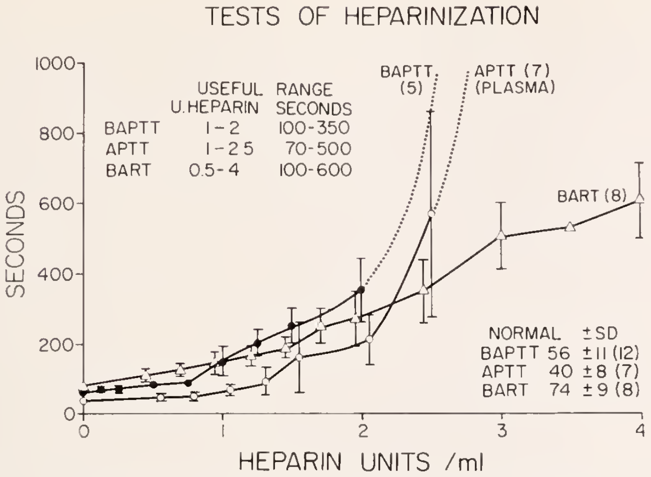

Fig. 6. Several methods of determining heparin level are compared. APTT: activated partial thromboplastin time using plasma. BAPTT: the same using whole blood. BART: whole blood activated recalcification time. Fresh human blood was prepared with serial heparin dilutions and tested by one or more methods using the BioQuest Fibrometer®. Numbers in parentheses indicate numbers of blood samples tested. The useful range of the APTT and the BAPTT is restricted to concentrations of heparin below about 2 units per ml because larger amounts unduly prolong these tests. The BART appears more satisfactory and, in addition, the reagent is stable at room temperature. BART reagent courtesy of BioQuest, Inc., Cockeysville, Maryland.

sure respirators and, more recently, PEEP has extended the effectiveness of mechanical respiratory support (4). Nevertheless, on occasion, low $Pa_{O_2}$ values and lung compliance become progressively worse with time as atelectasis, consolidation, and even oxygen toxicity increase. High inflation pressures may lead to the dangerous complication of pneumothorax. There is no way to rest the lung and, in consequence, patients with potentially reversible pulmonary lesions still die.

A few patients may be salvaged by the judicious use of hyperbaric oxygenation (6) but this is available in only a few centers and it increases the dangers of oxygen toxicity (7). Although hypothermia, by reducing metabolic needs, can theoretically compensate for reduced pulmonary function, this modality has not been applied with much success in acute respiratory insufficiency. Membrane lung support has now been shown to be possible for five to ten days during which mechanical ventilation and high inspiratory oxygen values ($FI_{O_2}$) can be moderated. The problem is thus when and how to institute extracorporeal perfusion.

If, in the course of acute potentially reversible pulmonary insufficiency, the patient shows hypoxic cerebral or cardiac deterioration despite knowledgeable respirator support, the desirability of perfusion should be considered. It is not possible to state absolute figures for the minimum $Pa_{O_2}$ or the maximum $FI_{O_2}$ or inflation pressure. Values of 35 to 45 mm Hg $Pa_{O_2}$, 70–100% $FI_{O_2}$ for longer than 24 to 48 hours, or more than 45 to 60 cm of $H_2O$ inflation pressures all offer special dangers to the patient and frequently signal treatment failure.

Where possible, the perfusion team should include members of the acute respiratory care team as this will do much both to prevent premature use of the membrane lung and to encourage its timely consideration. In addition, expert respirator care continues to be necessary during the period of perfusion. It is most desirable to institute perfusion, if it is to be done, before there is a pneumothorax, severe oxygen toxicity, or brain damage from hypoxia. Although prolonged perfusions can be carried out in patients with one or more of these complications, the prospect of salvage is greatly reduced (2).

Clearly, the membrane lung is no panacea and, in fact, there is already evidence that significant internal bleeding may be a contraindication to its use. Successful reversal of pulmonary pathology has been obtained in patients with intracranial bleeding although they subsequently died of brain damage (2, 8). Bleeding may have been a contraindication in the present case, although the central nervous system problem was thought initially to be due to an embolic complication not a hemorrhage. But for this, the patient had a good chance of recovery. In the present state of the art, bleeding is certainly the most dreaded complication of perfusion and almost certainly aggravated preexisting brain damage in this case. Better control of heparinization may change the situation, however (Figs. 5 and 6).

As expected, hyperbaric oxygenation effectively reversed the patient's pulmonary edema (9) but improvement in pulmonary function was insufficient to permit the hyperbaria to be discontinued. Cardiac failure, unfortunately, prevented the use of PEEP. It appeared clear that survival was not possible without the addition of perfusion. In the presence of the low cardiac output, the hyperbaria was most helpful in providing larger amounts of oxygen through the extracorporeal lung (Fig. 4), a circumstance permitting a lower $F_{IO_2}$ and so reducing the danger of oxygen toxicity. On perfusion, there was a prompt improvement in the cardiovascular status of the patient which permitted institution of PEEP. Improvement in dynamic compliance followed immediately, and the vicious cycle of the patient's pulmonary and cardiovascular deterioration was interrupted. It would certainly have been less satisfactory to initiate perfusion without the hyperbaria as this greatly enhanced gas exchange. On the other hand, without the availability of a hyperbaric chamber, perfusion would have been started perhaps 24 hours sooner and this might well have been beneficial. In addition, oxygen toxicity, suggested by the postmortem pulmonary findings, might have been avoided or minimized by a shorter period of hyperbaria.

PEEP can dramatically raise the $P_{aO_2}$ for any given $F_{IO_2}$. It appears to work by increasing the functional residual capacity by opening collapsed alveoli and combating increased surface tension forces tending to close them (4). Ordinarily hypotension precipitated by PEEP is associated with a reduction in cardiac output caused by diminished vascular volume and impaired venous return. It may usually be overcome by increasing the blood volume and is seldom a problem (4). In the present instance, there was a critically low cardiac output already despite a maximum increase in blood volume. Attempts to use PEEP before perfusion, therefore, aggravated the low cardiac output and deepened the shock. After perfusion was started, volume was given up by the patient to the circuit signaling reduction in the cardiac failure and PEEP became possible.

Major prerequisites for satisfactor long-term perfusion are:

1. A satisfactory membrane lung perfusion system. So far, the Bramson, General Electric-Peirce, Kolobow, and Landé-Edwards lungs have all been used for periods longer than five days. Nonmembrane equipment is not satisfactory. Semi-automated pumps are most helpful as it is virtually impossible to maintain the close surveillance of the perfusion ordinarily practiced in open heart surgery.
2. A sufficiently large and well-trained team, consisting of physicians trained in respiratory therapy, perfusion, and surgery, perfusionists, nurses, and laboratory personnel, so that continuous coverage may be provided.
3. Continuing knowledgeable respirator support. As indicated, it is very desirable to have the team closely associated with or part of the intensive respiratory care team. The perfusion reduces the total oxygen and carbon dioxide exchange need through the natural lung but does not eliminate the need for its function.
4. Equipment and techniques for the proper management of prolonged heparinization.

Other hematologic problems may develop and most teams have found that they need to have the ability to manage these themselves.

## Summary

1. Venoarterial perfusion with a membrane lung provided prolonged respiratory and cardiac support.
2. The use of hyperbaric oxygen provided stopgap support permitting assembly of the perfusion team, and stabilizing vital functions during initiation of perfusion.
3. The concomitant use of hyperbaric oxygen was helpful since oxygen transport was approximately doubled by virtue of the dissolved oxygen despite the low extracorporeal flow.
4. It was necessary to continue respirator support and the natural lungs provided about half of the oxygen exchange. Cardiovascular improvement resulting from the venoarterial perfusion permitted positive end expiratory pressure (PEEP) to be used.
5. The severe respiratory pathophysiology was reversed by the combination of perfusion and PEEP. Marked improvement in respiratory function was shown by an increased dynamic pulmonary compliance and it was possible to discontinue the perfusion.
6. Troublesome bleeding from a tracheostomy was controlled despite heparinization when the activated partial thromboplastin time was maintained at 3 to 6 times normal.

7. The patient had evidence of brain damage before perfusion and eventually died a cerebral death. One kidney was used as a transplant and has functioned well for nine months.

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# Discussion

Selected discussion is included because it adds important clinical information not otherwise available, or supplies the valuable opinions of an authority that might be irreplaceably lost. The discussion is brought together in one place, but it is arranged by subject in the general order of the symposium. *Editor's note.*

## *Membrane Lung Studies*

Our experience with membrane lungs includes laboratory studies using the Bramson, Landé-Edwards, General Electric-Peirce, and Travenol membrane lungs. We have used standard two-roller pump circuits in either venoarterial or cardiopulmonary bypass configurations. The venoarterial bypass system was used in evaluating the oxygenating efficiency of the General Electric-Peirce 1.0 $M^2$ and the Travenol 1.5 $M^2$ membrane lungs. With the General Electric-Peirce lung, at a bypass flow of 0.75 L/min/$M^2$, the outlet saturation was 95.7% and the oxygen transfer 28.8 ml/min/$M^2$. At a bypass flow of 1.0 L/min/M, the outlet saturation was 89.3%, but the oxygen transfer increased to 38.6 ml/min/$M^2$. Data from a Travenol 1.5 M lung showed that at a bypass flow of 0.835 L/min/$M^2$, the outlet saturation was 96.7% and the oxygen transfer 31.4 ml/min/$M^2$. At a bypass flow of 1.0 L/min/$M^2$, the outlet saturation was 91.2%, but the oxygen transfer increased to 40.5 ml/min/$M^2$. Such data show that simple oxygen transfer values do no appropriately describe the oxygenating capability of membrane lungs. As Dr. Galletti proposed, the use of rated blood flow more accurately reflects oxygenating capability. In order to have a uniform method of evaluating the membrane lungs available, the rated blood flow data should be determined. Rated blood flow is defined as the normothermic flow through an oxygenating device which will result in an outlet or arterial saturation of $\geqslant$ 95% when the device is presented with blood having an inlet or venous saturation of 60–75% with a hematocrit of 40 to 42%. Metabolic data were obtained in normothermic cardiopulmonary bypass in five 4 to 6 Kg puppies for two hours at flows of 2.7 L/min/$M^2$ through a General Electric-Peirce membrane lung. All animals survived the perfusions and one animal died on the fourth postoperative day of undetermined causes. The remaining animals were sacrificed 7–10 days following operation for histologic examination of the heart, lung, and kidneys. There was a significant increase ($p < 0.05$) in the venous and arterial $P_{CO_2}$ during bypass and in the first hour following bypass. At the time of sacrifice these values were not significantly increased when compared to preperfusion (control) values. Free plasma hemoglobin was elevated only to 39.6 mg% during bypass. The elevated leucocyte count after bypass was probably related to a normal postoperative leucocytosis. There were no significant morphologic changes in lung and kidney after bypass. The pathologist could not differentiate between the sections from the control animals or the bypass animal.

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My comments concern the Medical Devices Applications Program of the Heart and Lung Institute. There is new legislation being considered which may very well enhance the support available for the purposes of the Heart and Lung Institute and which may secondarily result in reorganization to so-called Bureau status. This will enable us to pursue avenues heretofore closed to us for financial reasons.

An annual report will be available shortly and newsletters are planned for more efficient dissemination of information about the Medical Devices Application Program.

There is now a reasonable opportunity to fund for periods greater than one year. These changes are a result of a memorandum Dr. Harmison prepared approximately a year ago. This will hopefully greatly facilitate performance of experimental work by providing a greater measure of continuity.

My own experience in this position has been a most gratifying one. Educationally it has been a superb experience, so that it has been possible for me to learn more in three or four months than I had learned for years before. It has become painfully obvious that we need men to work in the Branch who are known as sound investigators in this field if we are going to be maximally effective in establishing working relationships with sound investigators around the country. For this reason I have been authorized to recruit actively. At the moment, the Branch is looking for four types of individuals: 1. A hematologist with an interest in polymer chemistry; 2. An additional surgeon-physiologist; 3. An engineer who is a specialist in fluid mechanics; and 4. An electrical engineer with a special interest in instrumentation. As a means of adding attractiveness to the situation in the Medical Devices Branch, a rather careful exploration is being made of the possibility that a personal laboratory or clinical component be added to the activities of each person to be recruited. There appears to be some promise that some such development might come to pass.

Finally, the suggestion has been made to me that this might be an extremely valuable pursuit for someone who wishes to take a year of sabbatical leave from an academic position. I am convinced that had I taken such a sabbatical leave years ago and then returned to my Chairmanship of Surgery at the State University of New York, I should have been a far more effective Chairman than I was in fact. I hope those of you who have such possibilities in the offing will think very seriously about this possibility.

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### *Open Heart Surgery*

We have used the Bramson membrane oxygenator in over 500 open heart surgical cases since 1964. In general, our results in terms of gas exchange are similar to the data that has already been presented.

All membrane oxygenators that are used in routine open heart surgery have the following general characteristics:

1. They are disposable.
2. They are more difficult to prime.
3. They are more expensive.
4. They have constant volume.
5. They have low hemolysis.
6. They are intrinsically good filters.

Why should we switch to membrane oxygenators when the results using bubble oxygenators are already very good? The mortality rate with bubble oxygenators is no worse than with a membrane oxygenator. The only reason for routine employment of membrane oxygenators is based on the assumption that the biologic quality of the patient will be improved over that of patients treated on bubble oxygenators. Evidence for this assumption is still small, but if one can use a membrane oxygenator for days during prolonged perfusion with patient survival when a bubble oxygenator fails in the same test, one would assume that during a 120-minute perfusion a membrane oxygenator is more advantageous. Dr. Carlson of Cornell Medical Center has shown that patients perfused with a membrane oxygenator have superior psychological postoperative test results when compared to patients perfused by the bubble oxygenator. We have been unable to show improvements by psychological testing, but also used less elaborate tests than the Cornell group.

We have been unable to show any reduction in the incidence of cerebral microemboli with the use of the Bramson membrane oxygenator. In recent years blood filters have resulted in the most dramatic reduction in microemboli and in the improved quality of the patient following surgery. The principle difficulty in establishing differences in membrane and bubble oxygenators is that there are, to date, no established clinical standards by which to make comparisons. I am confident that membrane oxygenators will eventually be demonstrated to be superior to bubble oxygenators for routine use in the operating room. One of our objectives for the next few years is to evolve better and more sophisticated clinical tests to verify this assumption.

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### *Assisted Circulation*

I have enjoyed these papers and wish to confine my remarks specifically to those of Dr. Giron and Dr. Landé. Dr. Giron mentioned the Senning cannula which was developed for left heart bypass. This was employed on 25 occasions on clinical patients. Some seventeen or eighteen were patients with massive myocardial infarction and shock. In that group there were no survivors. My associate, Dr. Richard Cappelletti, has been more alert to the possibilities of this apparatus than I and has used this on a series of patients with a somewhat better outlook, but who would not have survived without. One of these was a patient with acute left heart failure without myocardial infarction. He did nicely and recovered. Three patients had abdominal explorations for emergency situations in the presence of left ventricular insufficiency which would otherwise have precluded laparotomy. These patients survived and did nicely. One additional patient lived a day after myocardial infarction, shock, and left heart bypass, but died at that time.

The reason for abandoning left heart bypass in myocardial infarction with shock was not any uneasiness about the adequacy of the method. The reason was simply that we failed to have access to patients until such time as survival appeared to the cardiologist to be out of the question. The last two patients did not come to be on left heart bypass until after the electroencephalograms had become flat. It is in my opinion not wise to think about this as a failure of resuscitation, because what was in fact necessitated was resurrection. Continuance under these circumstances appeared likely to accomplish nothing except to debase the whole thesis of mechanical support for the circulation.

It was because of the tactical difficulty of using an invasive method under these circumstances that my associates and I undertook external compression counterpulsation and worked upon it for several years. It was abandoned because the likelihood appeared to me too small that we should have decisive benefit from this, particularly in the presence of diffuse vascular disease which would be expected to minimize the flexibility of the peripheral arteries. Dr. Harry Soroff and Mr. Clifford Birtwell have pursued this avenue, having started it at about the same time that my associates and I did, and they have added several components which have made the method far more effective than our laboratory had succeeded in doing. This has a potential, therefore, to prove successful without alarming the cardiologists.

I should add that the original patient placed upon mechanical circulatory support for myocardial infarction and shock recovered after but three hours of venoarterial pumping with oxygenation and now, fifteen years later, still runs his own retail store. The possibility of long-term survival with support alone is therefore demonstrated, even though it may prove necessary to employ surgical intervention on a substantial fraction of these people.

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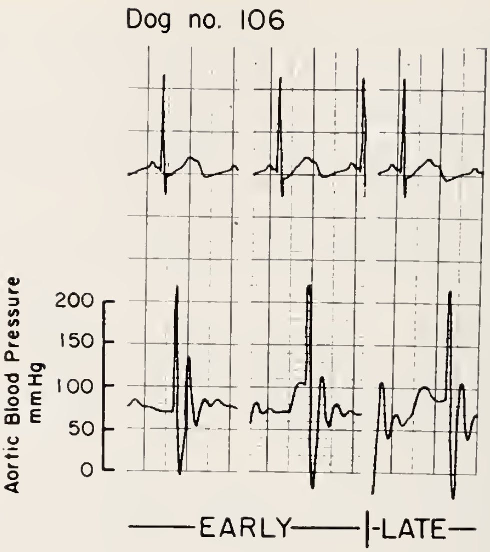
In response to the minimal patient survival reported following conventional treatment of patients in cardiorespiratory shock, my colleagues and I at the University of Minnesota, working under the direction of Dr. Richard C. Lillehei, have developed an assist unit capable of prolonged partial circulatory support. It is a self-contained assist device incorporating the modern concepts of synchronous pulsatile arterial inflow in conjunction with patient monitoring and mobility to allow for use of in-hospital facilities for diagnostic evaluation. In addition, rocking silicone rubber membrane oxygenators have been included to achieve a complete life support system.

This unit, soon to be incorporated into the clinical treatment armamentarium available at our institution, has been previously evaluated in the laboratory animal. Initially, dogs placed on partial venoarterial assistance with aortic route arterial infusion with three-fourths blood prime, developed early and sustained systemic hypotension, and subsequent death was attributable to homologous blood syndrome. After development of an appropriate preperfusion drug protocol our next six animals survived 24 hours of extracorporeal perfusion with successful one-month follow-up.

Data from these animals indicated the ability of this form of perfusion to maintain systemic pressures, a normal cardiac index, increased upper extremity and cerebral blood flow, and significantly increased urinary output. Venous lactate levels proved a reliable guide to successful perfusion. Arterial oxygen saturation increased, pH remained normal, and $P_{CO_2}$ decreased. Membranes received only 100% oxygen. At flows above 2500 ml/min., hypocarbia and apnea occasionally developed requiring endotracheal intubation and a controlled respiratory rate to prevent persistent atelectasis. Additional studies in sheep, where flows of up to 6 L/min could be obtained, indicated that the maximum oxygen exchange for the membrane units was 72 ml of oxygen/min/$M^2$. This peak value was seen whenever flows exceeded 2500 ml/min, indicating a fixed membrane capacity for oxygen transfer.

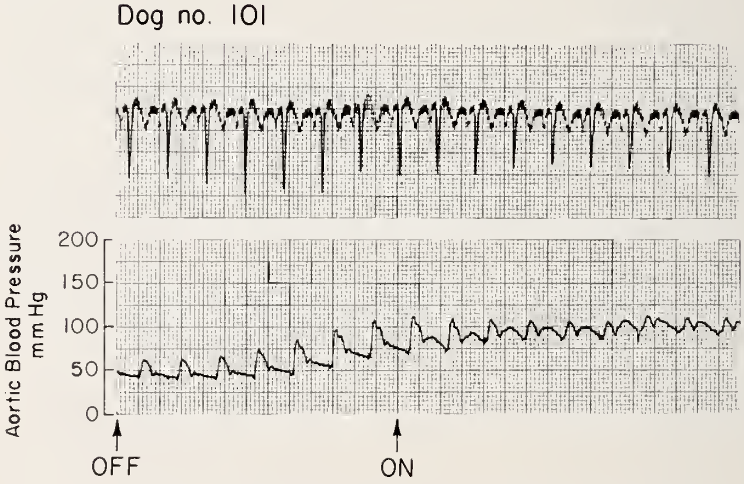
Typically, as compared to preperfusion control values, a significant decrease in hematocrit occurred ($p < .025$), with an early postperfusion return toward control levels without additional blood tranfusions. Leukocyte counts remained elevated in the late postbypass period ($p < .01$), while platelets showed a characteristic decline. Plasma hemoglobin levels remained relatively low, even when sheep were used at flows of 5 to 6 L/min and rarely exceeded 30 mg%.

Preliminary early experiments indicated that the synchronization system allowed us to manipulate the diastolic pressure response at will (Fig. 1). A pressure catheter, adjacent to the aortic root infusion cannula, was used to obtain these tracings which depict the arterial infusion wave as a positive and negative deflection occurring either too early or too late in reference to the animal's systolic pressure wave. An example of satisfactory synchronization with a characteristic elevation of the diastolic pressure is seen in Figure 2. Of note is the prevention of an elevated end-diastolic pressure which would result in greater oxygen utilization by the heart. The rapidity and extent of the decrease in diastolic pressure reflects the period of pump systole and the "runoff" characteristics of the peripheral vascular system; that is, the presence of vasodilatation and a patent microcirculation. The infusion pump is hydrauli-



*Flow: 1000 cc/min*
*Duration of pumping: 3 ½ hours*

Fig. 1.



*Flow 1000 cc/min. Duration of pumping 4 hours*
*Example of satisfactory synchronization*

Fig. 2.

cally driven, capable of up to 11 L/min flow and consists of vertical push rods which compress a vinyl pump head against a backing plate to effect ejection. An egg-shaped eccentric cam, attached to a reciprocal hydraulic motor, activates the rods. The system is triggered by the T-wave of the electrocardiogram with an adjustable delay mechanism.

In view of the above, we believe that our support system represents a useful possible adjunct in the care of patients with severe cardiorespiratory distress.

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### *Respiratory Insufficiency*

Adult respiratory insufficiency may be defined in terms of ventilatory failure with acute anoxia, with acute hypercapnia and acidemia, or with both. One must distinguish between those patients in whom anoxemia is paramount and exists even in the presence of effective alveolar ventilation. In many instances, oxygen therapy with control of secretions is the most appropriate treatment. Anoxemia due to diffuse disease of the lung, such as fibrosis and scleroderma, is best treated with oxygen and supportive therapy, since prognosis is ordinarily grim.

It is important to recognize that the anoxic and hypercapnic states are invariably accompanied by other derangements, especially pulmonary hypertension and heart failure. Ordinarily, rapid return of gas exchange to normal levels has proved to be the most effective way of treating pulmonary hypertension associated with ventilatory failure. In some instances, air-trapping mechanisms may induce a mechanical form of pulmonary capillary hypertension; inappropriate use of respirators may contribute to this. The group of patients with progressive heart failure (requiring monitoring of cardiac output and end-diastolic pressure) may be the best candidates for membrane lung gas-exchange maintenance.

Multiple microembolization of the pulmonary vascular bed is theoretically an indication for membrane gas-exchange support, but the progressive pulmonary hypertension and fibrosis of lung makes recovery prognosis so poor that this is questionable. Therefore, massive solitary embolization might be the ideal indication for this technique.

Postthoractomy (e.g., lung resection in borderline patients with chronic obstructive airway disease) should be considered as a potential area for membrane gas-exchange maintenance. Dr. Hill and others have shown that short-term support is feasible. Shock lung, pulmonary edema, burn lung, and gas-poisoning lung would also be theoretically ideal clinical states for membrane gas-exchange techniques. It must be emphasized, however, that the majority of patients can be adequately treated by conventional means, or with respirator support.

As a clinical lung physiologist, I am concerned, however, with the distribution of surface tension and viscous forces whenever the lung is "put to rest." Mechanical ventilation may be needed intermittently to maintain patency of airways, More fundamental studies of the effect of membrane lung techniques on surface films, and on lung mechanics is urgently needed before this technique should be widely used.

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Our patient was a 46-year-old male truck driver who became ill three weeks prior to his admission with symptoms of an upper respiratory infection. These symptoms progressed gradually in severity so that the day of his hospital admission he had paroxysms of coughing leading to deep cyanosis and collapse. The arterial oxygen tension on admission was 33 mm of mercury and following a tracheotomy, diuresis, phlebotomy, use of salt-poor albumin, steroids, and 100% oxygen delivered by a volume respirator, the arterial oxygen tension elevated to 36 mm of mercury. Two hours after tracheotomy, the oxygen tension had dropped to 28 and an hour later to 27 mm of mercury, in spite, of maximal therapy. For this reason, veno-arterial perfusion using the Landé-Edwards membrane oxygenator (two 3 meter units) was instituted with the venous cutdown into the iliac vein via the saphenous vein and the arterial cutdown into the innominate and brachial arteries via an axillary artery cutdown. The oxygen tension level in the blood rose to 33 mm of mercury and remained in this range over the next 48 hours of perfusion and ventilation with an MA-1 respirator delivering 1,000 ml of 100% oxygen at 35 cm of water pressure, 18 to 22 times per minute. In order to reduce the oxygen concentration in the respirator, the second venous line was inserted, thus increasing the perfusion flow from 1.3 to 2.6 liters per minute. At this point, the oxygen tension rose to the range of 45 to 50 mm of mercury, enabling us to reduce the oxygen concentration in the respirator from 100 to 80 and finally to 60%. Later, progressive reduction in the flow was possible with ultimate decannulation 62 hours after initiation of perfusion.

The patient continued to require the volume respirator over the next 14 days but with oxygen of only 60% concentration. The paroxysms of coughing required 7.5 mg of morphine intravenously every other hour interspersed with 5 mg of diazepam on the alternate hours for a period of 15 days. At the end of this time, the paroxysms subsided and the narcotics were withdrawn. The patient awakened and was gradually weaned off the respirator. He was discharged on the 37th hospital day weighing 115 pounds (weight on admission, 155 pounds). Two months after discharge, he returned to work as a truck driver and has been doing so without any disability. His electrocardiogram presently is normal as are his blood arterial gas levels. The titer for the Influenza A viral pneumonia rose significantly between the first day and the 27th day.

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Because of the availability of the Hyperbaric Chamber for the treatment of intractable acute respiratory disease, many patients have been sent from other institutions to The Mount Sinai Hospital. Some of these patients were salvaged, but many died, either because of the acute presenting lung disease or superimposed oxygen toxicity. Of thirty patients treated in our intensive care unit and/or the hyperbaric chamber only eight survived. Postmortem examination was performed in thirteen of the twenty-two who died and in twelve of these, the pathologists described changes in the lungs consistent with oxygen toxicity.

The adverse effects of prolonged exposure to high concentrations of inhaled oxygen is well known. Yet, in order to sustain life, the clinical situation demands high inspired oxygen concentrations for prolonged periods of time. Thus, we are faced with the paradox that the life-threatening hypoxemia requires high levels of oxygen and this treatment, as demonstrated by postmortem evidence, is then frequently either a contributory or a primary cause of death.

The clinical diagnosis of pulmonary oxygen toxicity is extremely difficult to establish. In some patients subjected to high oxygen exposure, the original lung lesion showed evidence of clearing, while a new diffuse alveolar infiltrate presumably due to oxygen toxicity was developing. As our experience with these patients increased, the $\mathrm{F_{IO_2}}$ was reduced and we accepted arterial blood levels which would have been previously unacceptably low. When the reduction of $\mathrm{F_{IO_2}}$ was followed by improvement in the radiographic pattern, we concluded that our assumption that oxygen toxicity had occurred was correct. This was noted in six of our patients.

The radiologic lesions appeared as diffuse bilateral pulmonary densities which were irregular in shape. Continued exposure to oxygen was accompanied by extension and coalescence of infiltrates until, in the most severe cases, almost the entire lung was densely opacified. The relatively nonspecific nature of the radiologic changes in a patient in whom there are likely to be several different causes for the development of diffuse bilateral pulmonary densities adds to the diagnostic dilemma. These include pulmonary edema, aspiration pneumonitis, bacterial and viral pneumonia, alveolar proteinosis, and extensive pulmonary embolism due to clot or fat. In fact, in all of our patients, at least one of these conditions existed prior to the administration of oxygen therapy and produced the presenting respiratory failure that necessitated prolonged high level oxygen treatment. Therefore, correlation between clinical and laboratory findings and accurate knowledge of the inspired oxygen content and length of time of administration is necessary to make a presumptive diagnosis of oxygen toxicity.

With less than one-third of our patients surviving, we sought a means of supplying the required oxygen without exposing the patient to the additional hazard of pulmonary oxygen toxicity. It became apparent that the only likely source of additional support for this terminal group of patients would be an extracorporeal oxygenator or "artificial lung."

Venovenous perfusion is a means of affording respiratory support during the acute phase of functional impairment. The principle potential advantage of venovenous bypass over venoarterial bypass is negligible hemodynamic disturbance. In this type of partial perfusion, the pump oxygenator is placed in parallel with the normal circulation, and prepulmonary oxygenation of venous blood is attained. This technique requires simple venous "cutdowns" and eliminates the need for thoracotomy and cannulation of the heart and major arteries. The membrane lung is suitable for long-term perfusion because it causes less blood damage than a conventional oxygenator.

The diffusion of oxygen across the membrane is dependent on the character of the blood film. Only the layer of blood in direct contact with the membrane becomes saturated. By increasing the pressure gradient for oxygen with hyperbaria this deficiency in transfer can be overcome.

The amount of oxygen actually transferred is a function of input desaturation and the Hg concentration, once a sufficiently high partial pressure gradient has been established. It has been shown that at pressures of 15 lbs or higher, the output of the oxygenator is always fully saturated whatever the input saturation, while in apneic dog experiments at 1 ATA, the output saturation progressively falls to unacceptable levels when the input saturation is below 60 percent even though the transfer is high (Fig. 1).

At present, function of a small membrane oxygenator is augmented by the simultaneous use of a hyperbaric chamber. The development of thinner silicone membranes, as little as 0.2 mils, the use of membranes as a pump, and more effective ways of overcoming the boundary layer by introducing turbulence into the blood may permit a wider clinical application of the membrane oxygenator at normal barometric pressures.

The clinical application of the combined use of the membrane oxygenator and

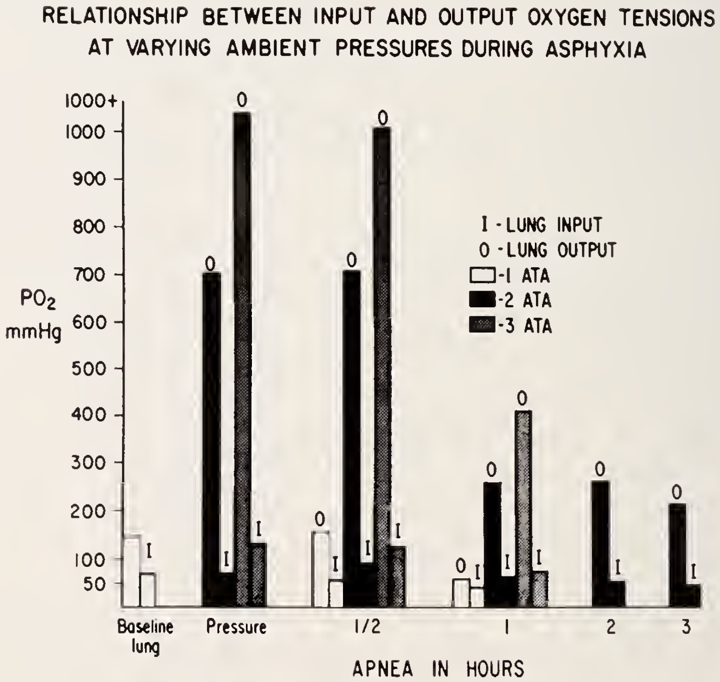

FIG. 1. Reprinted with permission from Anesth & Analgesia 49: 980, 1970.

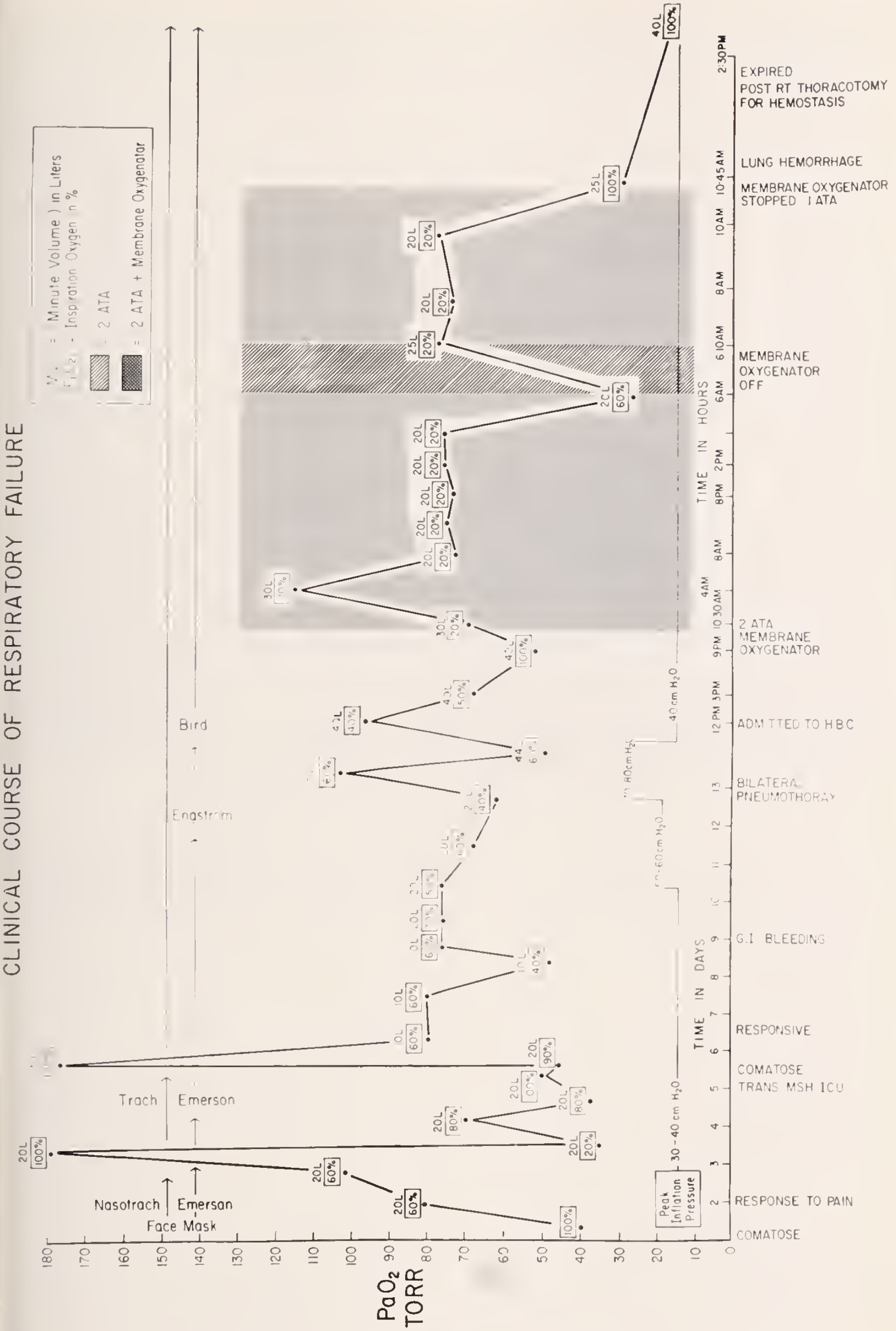
CLINICAL COURSE OF RESPIRATORY FAILURE
$\dot{V}_E$ = Minute Volume ) in Liters
$F_IO_2$ = Inspiration Oxygen in %
▨ = 2 ATA
▩ = 2 ATA + Membrane Oxygenator
PaO2 TORR
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Nasotrach
Face Mask
Emerson
Trach
Emerson
Engstrom
Bird
Peak Inflation Pressure
30 - 40 cm H2O
50-60 cm H2O
70-80 cm H2O
40 cm H2O
TIME IN DAYS
TIME IN HOURS
COMATOSE
RESPONSE TO PAIN
COMATOSE
TRANS MSH ICU
RESPONSIVE
G.I. BLEEDING
BILATERAL PNEUMOTHORAX
ADMITTED TO HBC
2 ATA MEMBRANE OXYGENATOR
MEMBRANE OXYGENATOR OFF
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FIG. 2.

the hyperbaric chamber is illustrated by the maintenance for 36 hours of a young male patient dying of respiratory failure. Figure 2 illustrates the course of this patient who developed an aspiration pneumonia following an overdose of heroin and was treated for four days with an $\mathrm{F_{IO_2}}$ of 80 to 100 percent via endotracheal tube and mechanical ventilation. His $\mathrm{Pa_{O_2}}$ was falling despite good conventional therapy and he became comatose. He was admitted to the Intensive Care Unit of The Mount Sinai Hospital, initially improved following vigorous physiotherapy and tracheal toilet, and regained consciousness. However, over the next nine days, a progressively higher minute volume, inflation pressure, and $\mathrm{F_{IO_2}}$ were necessary to maintain a satisfactory $\mathrm{Pa_{O_2}}$. On the thirteenth day he developed bilateral pneumothoraces and was transferred to the hyperbaric chamber for extracorporeal oxygenation. At 2 ATA using venovenous perfusion, he was placed on the membrane oxygenator. Once flow rates of 2000 ml/min were reached, his $\mathrm{Pa_{O_2}}$ remained at acceptable levels with pH and $\mathrm{Pa_{CO_2}}$ within the normal range. Minute volume and inflation pressure could be greatly reduced and ventilation was maintained with air (the equivalent of an $\mathrm{F_{IO_2}}$ of 40 percent at 1 ATA). After 24 hours bloody drainage from the right chest tube occurred. After 31 hours on the oxygenator, an attempt at weaning was unsuccessful. Bleeding from the chest became severe. A decision was made to take the patient off bypass, reverse the heparin with protamine, and perform an exploratory thoracotomy. Once off the oyxgenator, the patient's $\mathrm{Pa_{O_2}}$ dropped precipitously and he died. It is concluded that in patients for whom dangerously high levels of inspired oxygen, tidal volume, and inspiratory pressure are required to maintain oxygenation, earlier resort should be made to an extracorporeal source of oxygen.

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# Crystalloid Intracytoplasmic Inclusions in Lymphocytes of a Patient with Chronic Lymphocytic Leukemia

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## Introduction

In general, the ultrastructural features of peripheral blood lymphocytes of most patients with chronic lymphocytic leukemia (CLL) are similar to those observed in normal lymphocytes (1). Some differences have been found, however, in the average size of the cell and the cytoplasm, in the shape of the nucleus, and in the content of rough-surfaced endoplasmic reticulum (RER) (2, 3). Using electron microscopic cytochemistry, we have demonstrated a reduced number of lysosomes in CLL lymphocytes as compared to normal cells (4).

Intracytoplasmic inclusions, although a very rare abnormality, have been observed in blood lymphocytes of certain patients with CLL (5–10), other lymphoreticular disorders (11–13), and chronic lymphocytosis (14). These structures were morphologically distinguishable from inclusions reported in lymphocytes of certain normal individuals (15) and of a patient with rheumatoid arthritis (16). They had either a crystalline (5, 7, 8, 12–14) or a globular form (8–10) and in some instances were surrounded by membranes of RER (5, 8–10, 12, 14). The present report describes the morphologic findings in a CLL patient whose peripheral lymphocytes contained crystalloid intracytoplasmic inclusions, which were surrounded by membranes devoid of ribosomes.

## Clinical Data

A.E., a Caucasian female, was born October 20, 1915. Since age 25 she had a simple nodular goiter. At age 55, the patient noted gradual enlargement of cervical lymph nodes, weakness, and weight loss. A lymph node biopsy performed in September 1970, revealed infiltration with large lymphocytes which also penetrated the capsule into the surrounding connective tissue. In October 1970, the patient was admitted to the University of Ulm.

Physical examination revealed a nodular goiter, generalized nontender lymphadenopathy, and moderate hepatosplenomegaly. Laboratory studies including coagulation profile, liver function tests, serum electrolytes, and protein-bound iodine were within the normal range. Serum electrophoresis showed slight hypoalbuminemia and hypogammaglobulinemia, but no homogeneous spike (total protein, 5.3 gm% ; albumin, 3.16 gm% ; $\alpha_1$-globulin, 0.20 gm% ; $\alpha_2$-globulin, 0.56 gm% ; $\beta$-globulin, 0.66 gm% ; $\gamma$-globulin, 0.72 gm% ). Thyroid autoantibodies, indirect and direct Coombs' tests, and tuberculin test were negative. The hemogram was as

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follows: hemoglobin, 12.7 gm%; erythrocytes, 4.2 × $10^6/\mu l$; WBC, 22,600/$\mu l$; differential WBC: metamyelocytes 1%, band neutrophils 1%, segmented neutrophils 22%, eosinophils 1%, lymphocytes 72%, monocytes 3%; platelets 141,000/$\mu l$; reticulocytes 1.3%. Bone marrow histology revealed extensive infiltration with small lymphocytes with normal morphology and occasionally single lymphoblast-like cells with distinct nucleoli. There were only small residues of normal hemopoiesis.

The diagnosis of chronic lymphocytic leukemia was made, and cytostatic treatment was started with chlorambucil (6 mg per day). After 3 weeks, chlorambucil therapy was discontinued, and subtotal thyroidectomy was performed. Histologic examination revealed a thyroid adenoma. During the postoperative period, peripheral blood for electron microscopy studies and in vitro lymphocyte stimulation was obtained. One year later the patient presented with multiple osteolytic lesions which contained adenomatous thyroid tissue. Total thyroidectomy was performed, and histologic examination showed adenocarcinoma.

## Materials and Methods

Immunoelectrophoretic analyses using polyvalent and monospecific antisera were done according to the method of Scheidegger (17). Serum immunoglobulin concentrations were determined by single radial immunodiffusion (18).

Column-purified lymphocytes, prepared from heparinized blood samples taken on two separate occasions, were incubated in vitro with phytohemagglutinin (PHA) during a 7-day period. Details of the culture procedure have previously been described (19). $^3$H-thymidine incorporation into DNA of lymphocytes was determined according to the method of Naspitz and Richter (20).

For electron microscopy, column-purified lymphocytes prepared from heparinized blood (19) were fixed in 1.5% glutaraldehyde in 0.05 M sodium cacodylate containing 7.5% sucrose at 4°C. The cell pellets were postfixed in Millonig's phosphate buffered osmium tetroxide for one hour, dehydrated in graded concentrations of ethyl alcohol and in propylene oxide, and embedded in Epon 812. Thin sections were prepared with an LKB Ultrotome III, placed on grids, and stained with 1% aqueous uranyl acetate and lead citrate. The material was examined at primary magnifications from 3,000 to 40,000 in a Siemens 101 electron microscope.

## Results

### *Serum protein studies*

Immunoelectrophoretic analysis of the patient's serum showed reduction of IgG, IgA, and IgM corresponding to the hypogammaglobulinemia observed in cellulose acetate electrophoresis. No evidence for monoclonal gammopathy was found and Bence Jones proteinuria was not detectable. Serum immunoglobulin concentrations were IgG, 6.45 mg/ml, IgA, 0.45 mg/ml, and IgM, 0.42 mg/ml.

### *Lymphocyte response to phytohemagglutinin*

As shown in Figure 1, peripheral blood lymphocytes obtained on the third postoperative day after partial thyroidectomy, showed an extremely diminished

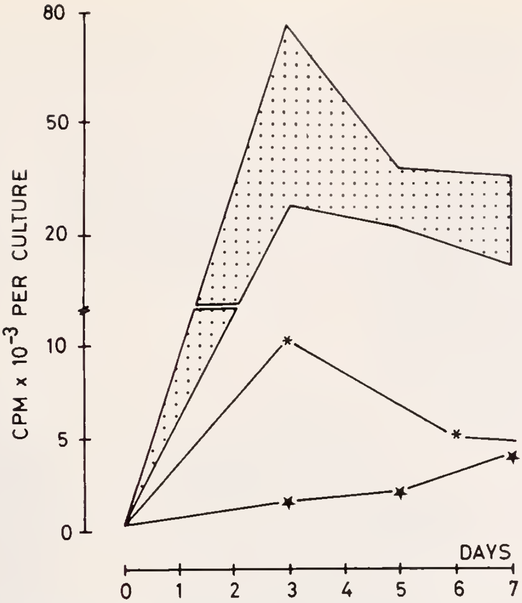


FIG. 1. $^3$H-thymidine incorporation during a 7-day incubation with PHA in vitro of peripheral blood lymphocytes from normal individuals (pointed area, mean ± S.D.) and from patient A. E. (★——★ 3 days and ✳——✳ 15 days after partial thyroidectomy). Values of control cultures are subtracted (We thank Dr. H. D. Flad, Ulm, for performing one of the PHA studies).

in vitro response to PHA with slight increase in $^3$H-thymidine incorporation on the 7th day of culture. Lymphocytes drawn on the 15th postoperative day showed a maximum response to PHA on day 3 of incubation in vitro. Although the time course of $^3$H-thymidine uptake paralleled the normal reaction pattern, the magnitude of the response was reduced in comparison to normal lymphocytes.

### *Morphologic observations*

In peripheral blood smears some lymphocytes showed small rod-shaped or rectangular cytoplasmic inclusions of varying size which were unstained by May-Grünwald-Giemsa. No other abnormal features such as vacuoles or Russell bodies were observed at the light microscopic level.

Electron microscopic study of the peripheral blood revealed that the majority of the cells were typical small lymphocytes. The nuclei of these cells were predominantly heterochromatic and nucleoli were frequently observed (1, 15) (Fig. 2). The cytoplasm generally contained numerous free ribosomes and occasional strands of rough-surfaced endoplasmic reticulum (RER). The Golgi zones were poorly developed; several typical mitochondria and rare lysosome-like organelles were usually present in the cytoplasm.

About 20 percent of the cell profiles contained large inclusions of intermediate electron density. These structures had an average width of 0.26$\mu$ and a length of 3–4$\mu$ (Figs. 2–8). They were comprised of linearly arranged strands which were approximately 100 Å wide (Fig. 8). The structures were generally square or rectangular in thin sections. They were surrounded by single membranes which were devoid of attached ribosomes (Figs. 5, 8). Some of these structures were in close proximity to the nuclear membrane complex (Figs. 7, 8).

A small population of cells was present which contained dumbbell shaped nuclei, often with a small component surrounded by a narrow cytoplasmic rim. The nuclei of such cells often showed areas of constriction (Fig. 9).

## Discussion

By light and electron microscopy, the occurrence of crystalline structures has been demonstrated in the cytoplasm of peripheral blood lymphocytes of a patient presenting as CLL, who later developed a metastasizing thyroid carcinoma. An increased incidence of malignancies in CLL patients is well known. Whether this association is the consequence of impaired immune surveillance mechanisms as in other immune deficiency syndromes (21) remains to be clarified.

In addition to other metabolic defects, the in vitro reactivity to PHA stimulation of blood lymphocytes from most CLL patients is diminished and/or delayed as compared to lymphocytes from normal individuals (22, 23). In this patient the lymphocyte response to PHA was decreased. The maximal $^3$H-thymidine incorporation occurred after three days of incubation, and the reaction pattern corresponded to that observed in normal lymphocytes. (The almost absent lymphocyte activation by PHA observed for cells obtained three days after partial thyroidectomy may be interpreted as the consequence of surgical trauma which may cause temporarily impaired lymphocyte transformation by this mitogen (24).)

The crystalloid intracytoplasmic inclusions in the lymphocytes of our CLL patient correspond to those recently reported in a CLL patient by Hurez et al. (7, 8). As in the present case, these crystalloids were bounded by a single membrane which was devoid of ribosomes. In contrast, comparable crystalline inclusions in the cytoplasm of CLL lymphocytes which were observed by Bernard et al (5), Bessis (6), and de Man and Meiners (12) were at least in part surrounded by RER. Similar crystals have been described in lymphoplasmacytoid cells of 2 patients with Waldenström's macroglobulinemia (11) and in plasma cells (25–29). The globular inclusions found in the cytoplasm of lymphocytes were in all instances bounded by membranes of RER (8–10) and thus had the morphologic characteristics of Russell bodies frequently observed both in normal and abnormal plasma cells (30, 31).

It has recently been reported (8) using immunofluorescence microscopy that the crystalloid, as well as the globular, inclusions in the cytoplasm of lymphocytes of certain CLL patients are IgM. Its structure also closely resembles that of a spontaneously crystallized monoclonal immunoglobulin (Ig) in the serum of a patient with multiple myeloma (32). In contrast, the inclusions recently reported in lymphocytes of a patient with chronic lymphocytosis are completely different (14). These were composed of bundles of tubular fibrillar material surrounded by RER and were non-reactive with fluorescein labelled

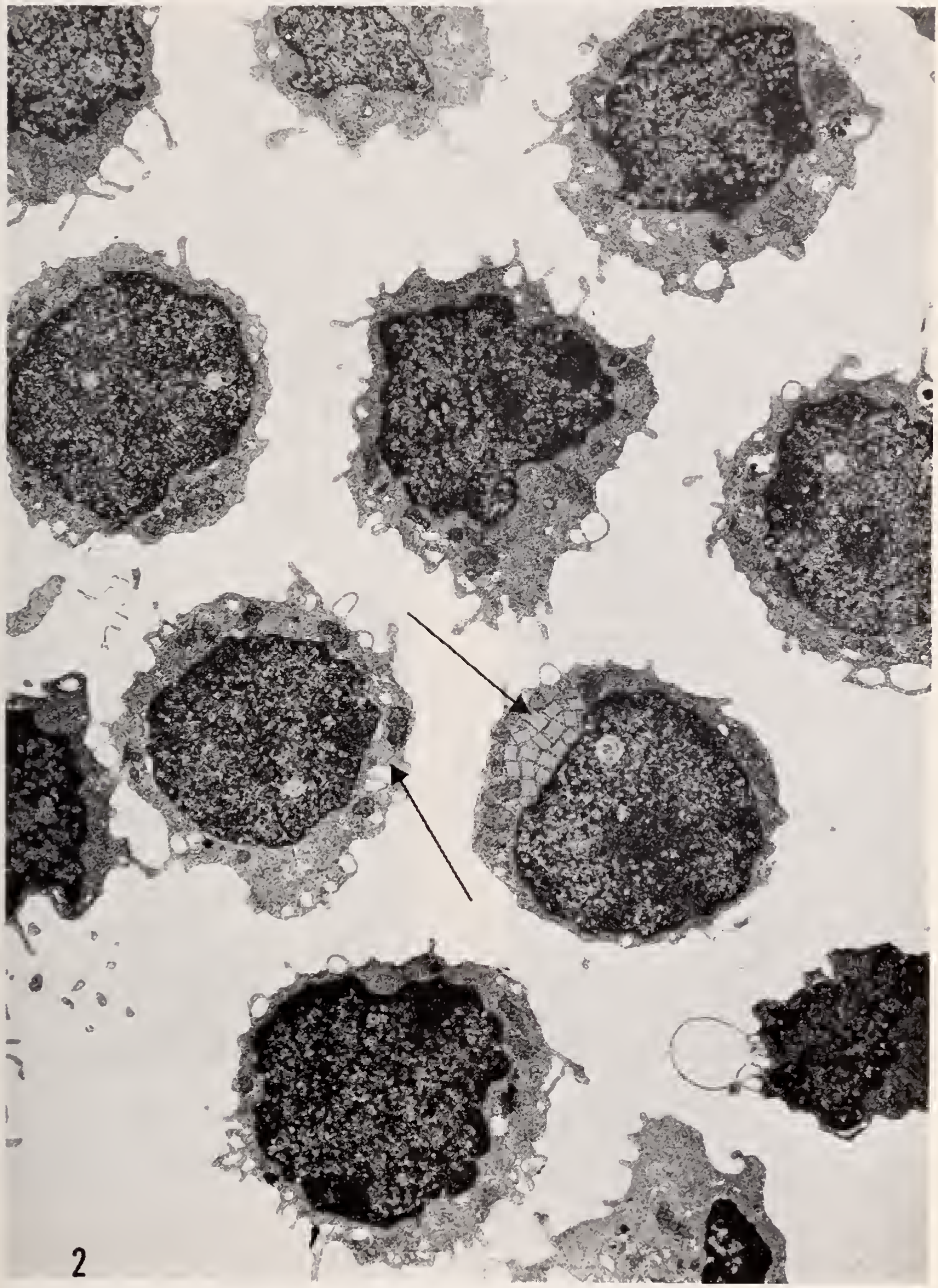
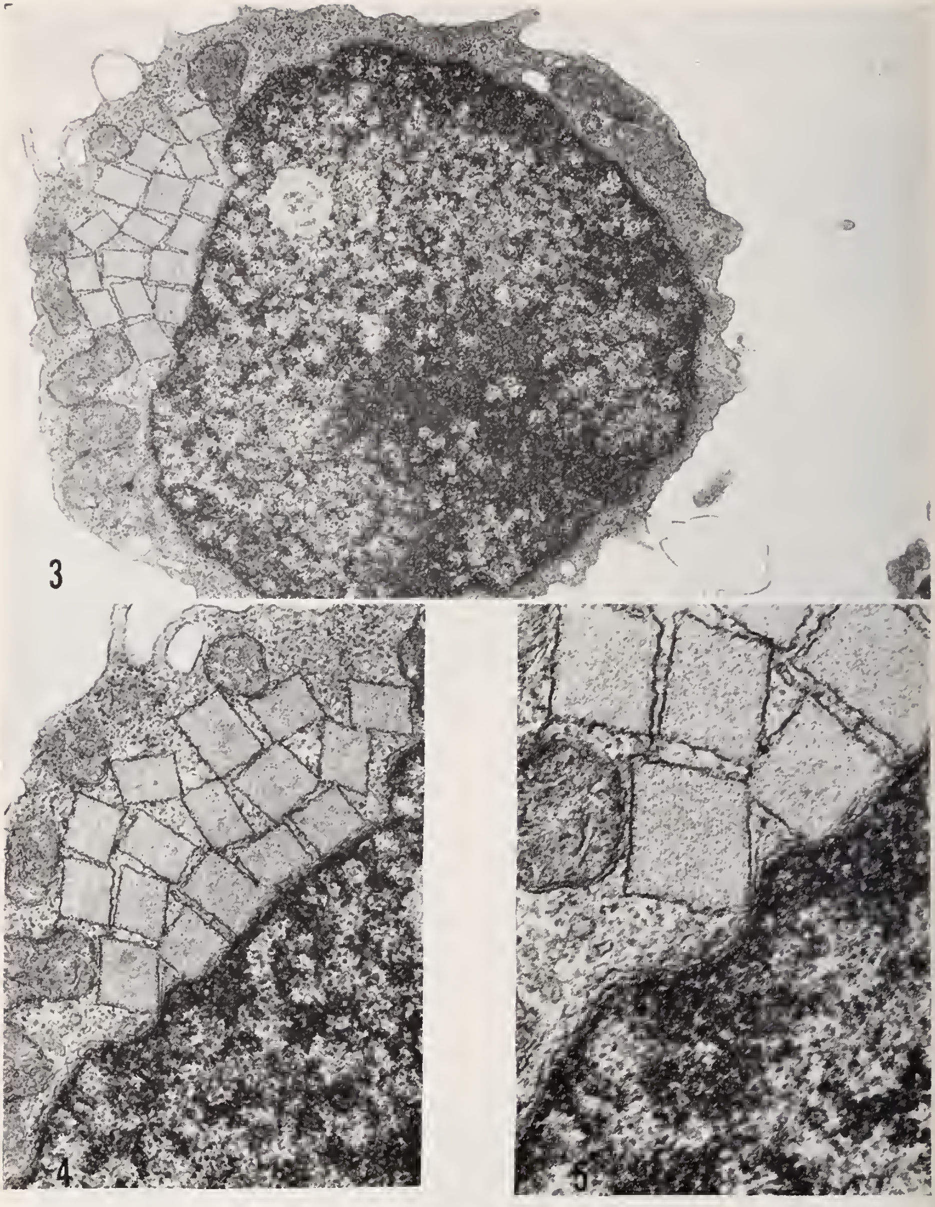


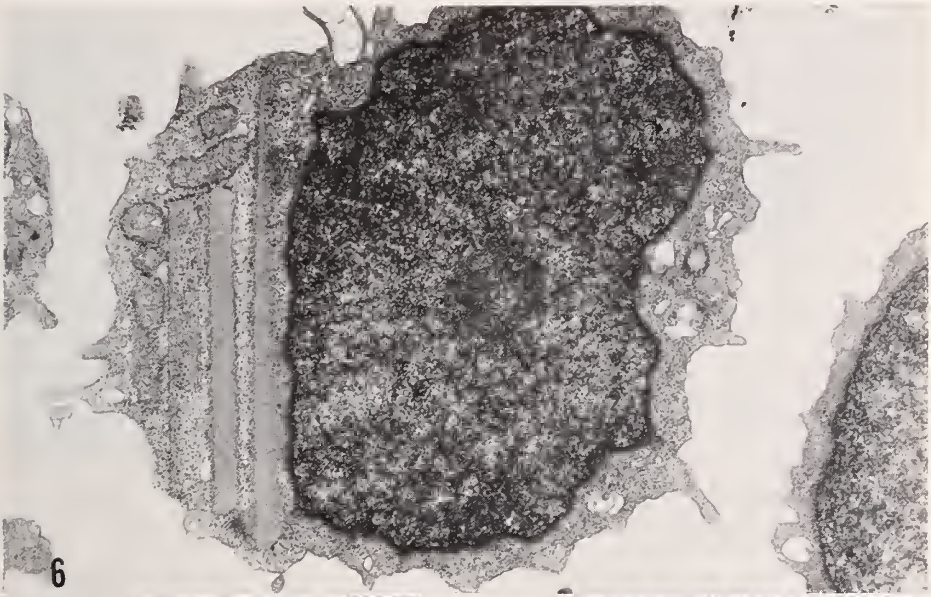
Fig. 2. Electron micrograph showing several peripheral blood lymphocytes. Two of the cells (arrows) show the characteristic inclusions. Mag. ×7,500.

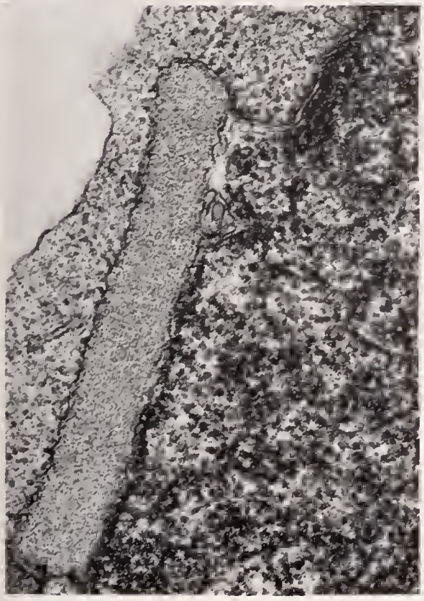


Figs. 3–5. Lymphocyte showing intracytoplasmic crystalloid inclusions of intermediate electron density. Fig. 3: Mag. ×20,000; Fig. 4: Mag. ×35,000; Fig. 5: Mag. ×70,000.

antibodies, suggesting that they did not contain immunoglobulin (14). In the present study it was not possible to characterize the crystalloids by immunofluorescence or other histochemical techniques, since the patient was not available for further investigation.

Resting lymphocytes contain only a few strands of RER (1, 15). Bone-marrow dependent (B) lymphocytes, however, are thought to be the precursors of antibody-producing plasma cells (33–35). A high density of Ig is detectable on the plasma membranes of the majority of blood lymphocytes from most





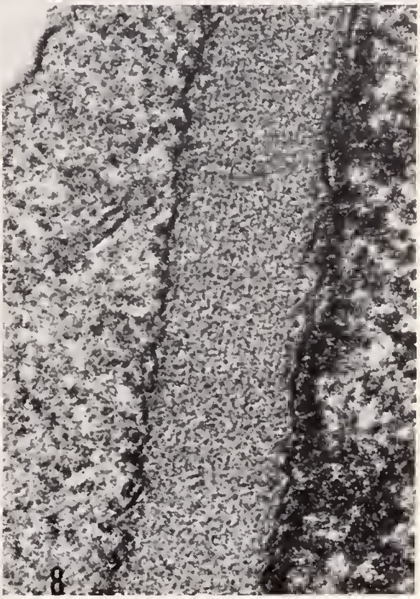


Fig. 6. Lymphocyte showing rectangular intracytoplasmic inclusions bounded by a single membrane devoid of ribosomes. Mag. ×20,000.

Fig. 7. Portion of another lymphocyte showing rectangular inclusion in proximity to the nuclear membrane complex. Mag. ×55,000.

Fig. 8. Higher power micrograph showing 100 Å strands with regular periodicity within a crystalloid inclusion. Mag. ×110,000.

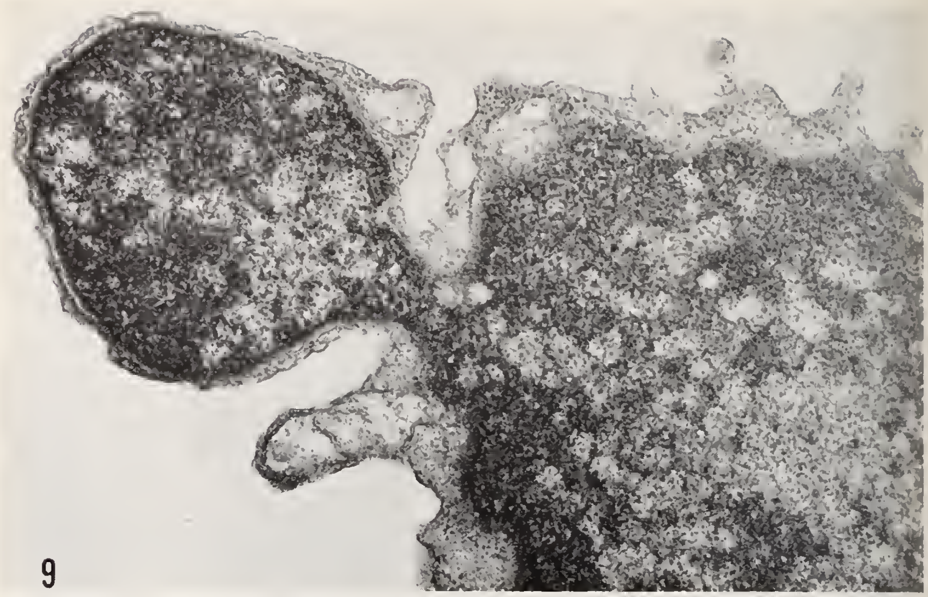


Fig. 9. Lymphocyte showing dumbbell-shaped nucleus with a smaller component surrounded by a narrow cytoplasmic rim. Mag. ×31.500.

CLL patients and this is considered to be a characteristic of B lymphocytes (36–38). Thus, CLL cells may have the capacity to differentiate into cells which synthesize Ig. Although Ig may be produced on free ribosomes, they are usually synthesized on ribosomes attached to membranes of endoplasmic reticulum (39–42). The proposed sequence of morphologic events is as follows (41, 42): After entering the cisternae of RER, Ig are transported to the Golgi complex and enclosed within membranes of Golgi vesicles; these post-Golgi vesicles migrate to the plasma membrane and fuse with it; and the Ig molecules are thought to be exteriorized by reverse pinocytosis. Whereas in plasma cells the Ig are released from the plasma membrane (42), in nonsecretory lymphoma cells they remain attached to it (41). If Ig formation, assembly, and secretion involves release of Ig from the RER to pinocytotic vesicles and their subsequent discharge via the plasma membrane, then intracytoplasmic crystalloid inclusions within Ig forming cells could result from altered kinetics at several possible steps. Although tentative, this interpretation would suggest that Russell bodies bearing ribosomes are the consequence of altered transport prior to release of Ig into Golgi-associated vesicles which lack attached ribosomes. This phenomenon appears to be a common occurrence both in normal Ig formation and in paraproteinemic states (30, 31). On the other hand, the abnormality which we have observed, namely, crystalline material surrounded by membranes devoid of ribosomes, could be the consequence of altered transport by the vesicular system. Studies of intracellular formation, assembly, and secretion of Ig by cells from patients with this albeit unusual morphologic abnormality are essential to establish this hypothesis and would be of possible importance to the investigation of the normal pathways of Ig production.

## Summary

Crystalloid intracytoplasmic inclusions are demonstrated in the peripheral lymphocytes of a patient with chronic lymphocytic leukemia who later in the course of the disease developed metastasizing thyroid carcinoma. These crystalloids are approximately 3–4$\mu$ long and 0.26$\mu$ wide, have a periodicity of 100 Å, are bounded by membranes devoid of ribosomes, and may be the consequence of abnormal immunoglobulin formation.

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# GUIDE FOR THE PROVISIONAL SELECTION OF ANTIBIOTICS

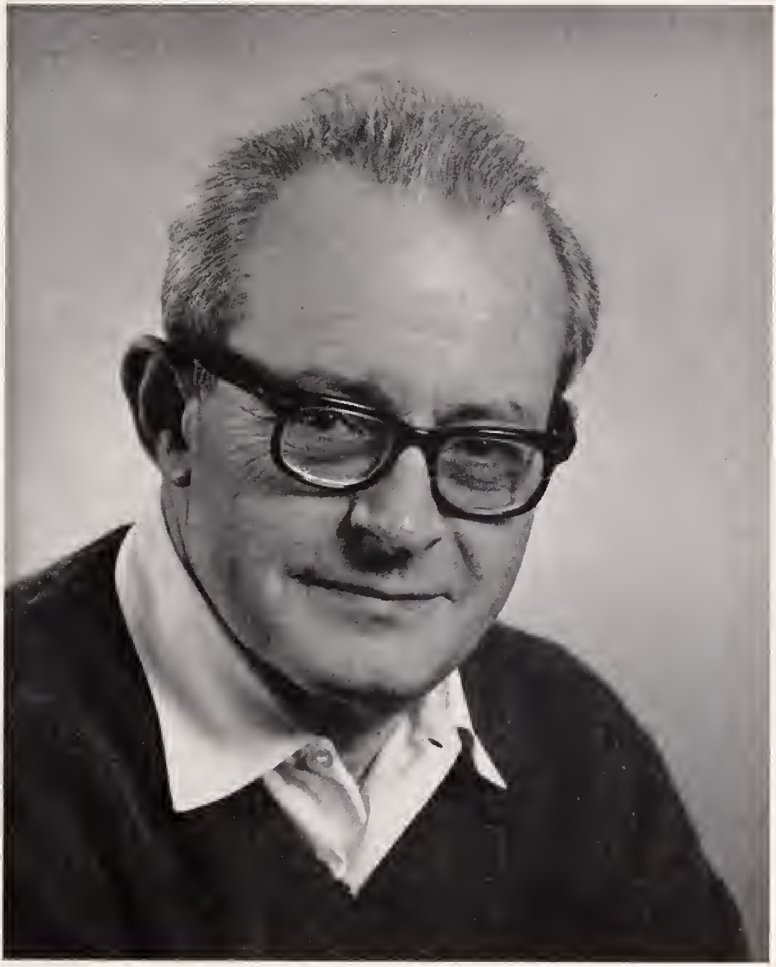
## (Pending Laboratory Sensitivity Determinations)

*The appended table represents a computer-derived summary of the total experience of the antibiotic susceptibility of microorganisms isolated at The Mount Sinai Hospital during 1971. Its use as a guide for the provisional selection of antibiotics at this hospital until laboratory sensitivity determinations are made is obvious. By extrapolation, it may serve as a guide to the practicing physician elsewhere.*

*As a service to our readers, it is our intention to publish these findings annually as soon as they are compiled.*

### COMPARATIVE ANTIBIOTIC SUSCEPTIBILITY OF MICROORGANISMS ISOLATED AT MSH IN 1972

| | DRUGS | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gram Positive Organisms | Ampicillin | Carbenicillin | Cephalothin (Keflin) | Chloramphenicol (Chloromycetin) | Erythromycin—Oleandomycin* | Gentamicin | Lincomycin | Neomycin—Kanamycin* | Penicillin | Oxacillin (Prostaphlin) Methicillin (Staphcillin)* | Polymyxin—Colimycin* | Streptomycin | Tetracycline | Vancomycin | Sulfonamide | Nalidixic Acid (Neggram)** | Nitrofurantoin (Furadantin)** |
| Staphylococcus Aureus | 4 | — | 1 | 1 | 2 | — | 1 | 2 | 4 | 1 | — | — | 2 | 1 | 3 | — | — |
| Strept. Fecalis (Enterococcus) | 2 | — | 5 | 1 | 3 | — | 6 | 5 | 2 | 6 | — | — | 5 | 2 | 6 | — | — |
| Beta Hemolytic Streptococcus | 1 | — | 1 | 2 | 1 | — | 1 | 6 | 1 | 2 | — | — | 3 | 1 | 6 | — | — |
| Streptococcus Viridans | 1 | — | 1 | 1 | 1 | — | 1 | 5 | 1 | 2 | — | — | 3 | 1 | 5 | — | — |
| Anaerob. and Microaeroph. Streptococci | 1 | 1 | 1 | 1 | 2 | 3 | 2 | 5 | 1 | 2 | — | 4 | 2 | 2 | 6 | — | — |
| Diploc. Pneumoniae (Pneumococcus) | 1 | — | 1 | 1 | 2 | — | 2 | 6 | 1 | 1 | — | — | 2 | 1 | 3 | — | — |
| Clost. Perfringens (B. Welchii) | 1 | 1 | 2 | 1 | 2 | 5 | 3 | 6 | 1 | 2 | — | — | 2 | 2 | 5 | — | — |



SOLOMON A. BERSON, M.D.
1918–1972

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# Introduction

DOROTHY T. KRIEGER, M.D.

It has been a year since Dr. Berson's sudden and premature death—a tragic loss to the Mount Sinai Medical Center and the international scientific community. We at Mount Sinai School of Medicine were fortunate to have had him as Chairman and Professor of the Department of Medicine for four all too short years. His dynamism, vision, intellectual clarity, intellectual and personal honesty, and encyclopedic knowledge and inspired teaching brought an atmosphere of scientific ferment to the department that permeated all its levels—professional staff, patients, house staff, and students. His scientific achievements, clinical expertise, empathy with patients and staff alike, were a model and goal for all who worked with him. He brought unceasing effort to all these facets of his character, and a desire for uncompromising excellence in the functioning of the department and of its members, for which he so well served as an inspiration. He had no patience with fuzzy thinking, scientific or otherwise, and his critiques sharpened the thinking of all who were involved in any discussions with him. It was soon learned that any interchange should be avoided without adequate preparation with regard both to content and concept.

My own association with him was both a professional and personal one. He was an individual of great warmth, loyalty, and understanding. I treasured our scientific conversations for their scope and insight, the questions they raised, the amount I could learn. Somehow, at their conclusion, they would wander afield to other areas: a flood of such instances comes to mind, such as the pigments Caravaggio used in his painting of Bacchus, the exemplary writing of Ford Maddox Ford, questions concerning the origins of the Etruscans, his unearthing of an unpublished Paganini score, the derivation of a word (usually settled by recourse to the Oxford English Dictionary), the meaning of a Pinter play, or the gastronomic and vintage offerings of certain culinary establishments. Sol was a great mathematician and chess player. These were interests I could not share with him. He was also a superb violinist, and I cherish the hours of playing of Beethoven, Mozart, and Haydn piano and violin sonatas together. Occasionally these would end with his saying that I was not always up to tempo; discussions with his other music-playing friends led to a consensus that in music, as in other things, Sol always did things quickly.

Shortly after his death, Mrs. Berson received and was most touched by a note from Dr. R. D. Ganatra of the Radiation Medicine Centre in Bombay, India, where Sol had served as a consultant in 1970, a stay which he looked back on with great fondness for the people he had met there. The note said, in part: "In

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the radioimmunoassay techniques that he set up, the hormone is assayed by the difference between the bound and unbound fractions. All of us who came in contact with him at the Radiation Medicine Centre have been infinitely bound to him by science, emotion, affection, and love, so that in our relationship with him, there is nothing unbound. And when that happens, in his assay technique, no estimate is possible of the quantity of hormone. So is his loss—inestimable and unassayable."

Together with Dr. Rosalyn Yalow, Dr. Berson made revolutionary contributions to numerous fields of medicine, and the techniques they pioneered have been extended to countless others. In their fundamental studies on the kinetics of circulating insulin, they appreciated the fact that their discovery of insulin antibodies could form the basis of radioimmunoassay technique. Utilizing the basic principles of such a technique and their own detailed knowledge of the physics and chemistry of this technique, they went on to establish such assays for other hormones, most notably growth hormone, ACTH, parathormone, and gastrin. The development of such techniques, however, was only a means for the initiation of studies designed to elucidate the physiology and chemistry of hormone secretion. Notable contributions were made in the fields of insulin, growth hormone, ACTH, thyroid, parathyroid, and gastrin secretion, as well as classic studies in the kinetics of albumin synthesis and degradation.

It must have been as a medical student that Sol read an article in *The Journal of The Mount Sinai Hospital* that he was particularly fond of quoting on medical student rounds. This was by Dr. Soma Weiss on "Some Observations and Psychologic Reactions of Medical Student A.S.R. to the Onset and Symptoms of Subacute Bacterial Endocarditis" (Vol. 8, 1079–1084, 1942). We of the Mount Sinai School of Medicine felt that the most fitting tribute we could offer to Sol's memory would be an issue of this journal devoted to contributions on subjects in his major areas of interest. His work in these fields has been a vital force in the studies of many prominent investigators in these areas. Therefore, we have asked those who were close to him both professionally and personally to contribute to this memorial issue. The nature and quality of their response is but another measure of the man.

# Curriculum Vitae

SOLOMON A. BERSON

*Born:* April 22, 1918
*Died:* April 11, 1972

*Degrees:*
B.S., 1938, City College of New York
M.Sc., 1939, New York University
M.D., 1945, New York University
D.Sc. (Hon.), 1966, Long Island University

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| 1939–1941 | Assistant in Anatomy, N.Y. University College of Dentistry |
| 1941–1942 | George Blumenthal Fellow in Anatomy, N.Y. University Graduate School |
| 1942–1945 | Lecturer in Physiology, Hunter College |
| 1945–1946 | Intern, Boston City College |
| 1946–1948 | 1st Lieutenant—Captain, MC, AUS |
| 1948–1950 | Resident, Internal Medicine, Bronx Veterans Administration Hospital |
| 1950–1954 | Radioisotope Unit, Bronx Veterans Administration Hospital |
| 1951 | Diplomate, American Board of Internal Medicine |
| 1954–1968 | Chief, Radioisotope Service, Bronx Veterans Administration Hospital |
| 1963 | Senior Medical Investigator, Bronx Veterans Administration Hospital |
| 1968 | Professor and Chairman, Department of Medicine, Mount Sinai School of Medicine of The City University of New York |
| 1968 | Director, Department of Medicine, The Mount Sinai Hospital of New York |

*Societies:*

Association of American Physicians; American Society for Clinical Investigation; American Physiological Society; Fellow, New York Academy of Sciences; Fellow, New York Academy of Medicine; Interurban Clinical Club; Harvey Society of New York; Endocrinological Society; American Diabetes Society; New York Diabetes Association; Society for Experimental Biology and Medicine; American Association for the Advancement of Science; American Medical Association; Fellow, Royal Society of Medicine; National Academy of Sciences (posthumous)

*Other Activities:*

Editorial Board, *Diabetes*, 1965–1967; Editorial Board, *Journal of Clinical Investigation*, 1958–1968; Editorial Advisor for *Diabetes Literature Index*, 1966– ; Editorial Board, *Hormone and Metabolic Research*, 1968– ; Editorial Board, *The Mount Sinai Journal of Medicine*, 1968– .

National Advisory Council, NIAMD, NIH, 1961–1964; Board of Scientific Counselors, NIAMD, NIH, 1966–1969; U.S. State Department American Specialist Grant Lecturer in South America, 1966; WHO Consultant Radiation Medicine Center, Bombay, India, November–December 1970; Scientific President, Second International Symposium on Protein and Peptide Hormones, Liege 1971; Councillor, Harvey Society, 1970; Advisor, Biological and Medical Sciences, American Peoples' Encyclopedia; Councillor, Argentine Diabetes Association

*Honors:*

*Honorary Memberships:* Diplomate of Pan American Medical Association; Peruvian Society of Endocrinology, Chilean Society of Diabetes and Metabolic Diseases; Chilean Society of Endocrinology; Argentine Society of Endocrinology and Metabolism; British Association of Clinical Biochemistry; Alpha Omega Alpha; Phi Beta Kappa

*Awards and Prizes:* Gairdner Award, 1971; Dickson Prize, 1971; Academy Medal, New York Academy of Medicine, 1971; Howard Taylor Ricketts Memorial Award, 1971; American College of Physicians Award, 1971; Medal of the Swedish Medical Society, 1970; N.Y. University School of Medicine Alumni Achievement Award for 1969; University Medal, University of Liege, 1968; Van Slyke Award and Medal, American Association of Clinical Chemistry, 1968; Distinguished Alumnus Lecturer, N.Y. University School of Medicine, 1966; Banting Memorial Lecture and Banting Medal, American Diabetes Association, 1965; First William S. Middleton Medical Research Award, 1960; First Eli Lilly Award of the American Diabetes Association, 1957; Harvey Lecture, 1966; and numerous other invited lectureships

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## Dr. Solomon A. Berson

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# A Personal Appreciation

JOSEPH ATTIE, M.D.

It is very difficult for me to think of Sol Berson as being gone. Since his death something very strange has been happening to me. During the two or three years before he died, we had grown further apart. He had assumed greater responsibilities, and we both were very busy. Our friendship was somewhat cyclic; we would be away or extremely occupied or he would be travelling or very busy and for that reason we only saw each other infrequently. It was one of those situations where, if you didn't see the man, you were always with him anyway; it didn't really matter, since he was your friend. But now that he is gone forever, you begin to realize how close you really were. I think I have talked about Sol a thousand times in the past few months. While he was alive, and during the past few years, if I mentioned him two or three times a year, it was only because something arose and it was essential to do it. Now I find that it is frequently essential to do so because something concerning him must be discussed. You always knew that Sol Berson was around when you needed his opinion on some matter. For example, although we had very little contact in our work, there were times when it was necessary to obtain information on a matter that no one else could provide. On routine questions relating to a subject, I probably would never have bothered him if he were alive, but now I find that I would like very much to get his opinion about many aspects of our work.

Last year my family and I traveled to Italy. While we were planning the trip, we mentioned it to Sol, and he said, "You're going to North Italy? That's great. I'll be right over to discuss it with you." We knew he was very busy at the time, but he came to the house and brought over a stack of books. He had them all marked, and he had maps that were all set up. He prepared an itinerary for us and told us what to see in each of the towns we were to visit. On our return, we were very embarrassed, because when we got to one of the towns he mentioned it was extremely hot, and we were tired, and decided not to stop there. I was ashamed to tell him this. He said, "What did you see in so and so?" I said, "Well, not too much," and I did not know how to apologize because if I told him we didn't stop there, he would have been a little bit peeved because he had outlined it so well for us.

I had known Sol Berson since we were in high school together and all through our college career. The memories of those days are very sketchy. Many of us do not keep diaries; we just have little spots in the brain which connect with each other as small links in a very long chain. I recall the first time that we got to know each other; it was the first time I was flattered by anybody. But, this was a common thing with Sol; he gave you credit for things you did not deserve. He started out by telling me, "Joe, I think you should be on the Math team." I

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really wasn't prepared or capable of it and I hesitated, but because it was a challenge I accepted it. He had promised to get me on the team and did so. After a few meetings, I realized that I was not prepared and devoted much of my last year in high school to studying the entire textbook of differential and integral calculus, just to be able to talk to Sol. I entered one math competition and it was only because Sol put me there, but I didn't quite make it. They allowed three minutes for a problem that I could not have completed in an hour. Sol Berson made you feel that you could accomplish the impossible and, consequently, you tried to do it. As a result of this experience, when I got to college, I did very well in math. But in truth I was not a good math student; I was only reflecting the things that Sol Berson kept putting in front of me to do and I kept doing them.

I recall the time we lived in Brooklyn, when he used to visit at my home. One day he came to the house and asked me if I wanted to play chess. As is well known, Dr. Berson was an expert chess player and I could never have hoped to play at his level. He wanted to even up the game rather than change his tactics and, consequently, he went into another room and played blindfolded through the window, calling out the moves and giving me a rook ahead. He always won anyway. It was very kind of him to play with me. It was the sort of kindness that persisted throughout his life. His behavior towards his students, his colleagues, and his friends always reflected the attitude that you were his equal in every pursuit.

One of Sol Berson's greatest interests was music; he was a fine violinist. I heard him play many times and it was always a wonderful experience. There were better performers, but none more passionate about it. When he heard music by the composers he loved best, whether the three B's or others, he became very excited. He would get up in the middle of a performance and start to walk around enthralled when the Gloria or the St. Matthew Passion or some other composition he loved was being played. Knowing this great passion about music and realizing that he did not get out very often and since we saw each other infrequently, I invited him on a few occasions to join me at a concert at the Bohemians (the New York Musicians Club). These concerts were attended mostly by professional musicians who belonged to the club. A performer would be invited to play when he was in town for some other concert. The musical program was not generally planned in advance. At the last minute I would call up Sol and ask him whether he would like to join me, and he would say, "Well I have to prepare a paper," or "I am writing this chapter," or "I'm really very busy, how long will this take?" I would answer him that the program would last an hour to an hour and a half, that the traffic at that hour was not too great, and that we could make the trip each way in less than a half an hour—I would give him an exact calculation. He would then ask "Will we be back at 10:45?" and I'd say "yes, probably." And he would agree to come on that condition. For two hours Sol would be completely relaxed. These great releases, whenever they occurred, whether it was at these concerts or some other function, were great for Dr. Berson, but did not occur often enough. This was one of his difficulties; he was so intense about perfection in everything, that such periods of relaxation were rare. When they occurred, however, they were complete. Even when he was listening to music, he would be completely wrapped up in it and enthralled. As in most areas, he would give the other person credit for being his equal or for understanding things the way he did. For example, he always would claim that you knew the music as well as he did, which of course you didn't.

Several years ago, we formed a Thyroid Club; it was composed of a group of physicians from different hospitals and in various specialties, but all interested in thyroid diseases. We had decided to meet outside the hospital milieu to discuss freely certain aspects of thyroid disease. I asked Dr. Berson whether he would join us at these meetings, and he said, "Okay, I'll come for your sake." This is the way he put it. We did have a tremendous amount of talent at these sessions, but it was obvious that whenever he discussed any of the problems, he presented the subject in a succinct and clear manner, getting right to the core of the problem. The discussion usually ceased at that point. I'm sure that those who have worked with Sol Berson, will all agree that no matter what he discussed in various subjects, he was able to capsulize it so that anyone could understand it. Many of the things he dealt with were beyond the understanding of many of us, but the way he handled the subject made it comprehensible to everyone. When he lectured or talked on a subject (and it was a pleasure and a privilege to listen to him) you felt that here at last you have learned something about a subject that you thought you knew something about. He added this dimension to almost any subject he discussed. It is for this reason that no matter what problem I attack now, and especially in the last few months, I find that getting it summarized and understanding it fully is very difficult without Sol around to help do it.

# Solomon A. Berson: The VA Years

ROSALYN S. YALOW, Ph.D.

The Radioisotope Unit at the Bronx Veterans Administration Hospital was initiated in 1947 by Dr. Bernard Roswit, then as now, Chief of Radiotherapy. While moonlighting from the Physics Department of Hunter College, I joined the unit on a part-time basis in December of that year and shortly thereafter work began in the application of radioisotopes to clinical diagnosis, therapy, and investigation. It seemed especially clear that the ready availability of radioisotopes would provide a torch to light the way in investigative medicine. Nevertheless, strange as it may now seem, the first internist associated with the unit resigned in January 1950 after six months' service, concluding that "there is insufficient stimulus and work in my present capacity to warrant pursuing it on a full-time basis." In seeking a replacement we turned to the Medical Service. Dr. Bernard Straus, then our Chief of Medicine and now holding that position at Beth Israel Hospital, suggested that we would be fortunate if we could attract Dr. Solomon A. Berson, who had proven outstanding and was to complete his residency at the VA in June 1950. Although other better known physicians were under consideration, we were indeed fortunate in choosing Sol and being chosen by him. Sol and I started to work together in the Spring and he joined our unit in July. Although he initially shared a part-time private practice, before long he found that the Unit provided sufficient "stimulus and work . . . to warrant pursuing it on a full-time basis."

Our early work together was on blood volume determinations and the thyroid. When our first major paper, on thyroidal and renal iodide clearances, appeared in the February 1952 *Journal of Clinical Investigation*, Sol was delighted to receive a note from F. R. Keating, the eminent thyroid expert, that he (Keating) ". . . regard[ed] it as the most important contribution to the problem of diagnostic tracer procedures which [had] as yet appeared."

In 1953, Sol was appointed Assistant Clinical Director of the unit and in 1954, when it became an independent full professional service, the first so designated by the VA, he became its Chief. Through the years, as his accomplishments and reputation grew, Sol received numerous offers for university-related positions. Invariably, the reply was an only slight variation of:

> "Your faculty includes a large number of distinguished colleagues and it would be an honor for one to be numbered among them. However, much as I find the climate of a medical school environment extremely appealing, the facilities in my own laboratory and the circumstances of my present position are so favorable that I am disinclined to consider making any change."

From The Solomon A. Berson Research Laboratory, Veterans Administration Hospital, Bronx, N.Y., and Mount Sinai School of Medicine of The City University of New York.

The 1950's and 60's were a period of immense expansion in medical research and a particularly exciting one in our laboratory. We worked in many areas, almost simultaneously—thyroid physiology, protein metabolism, radiation chemistry, and then in the discovery of the ubiquitous antigenicity of insulin in man, which led to the development of radioimmunoassay and the new fields it opened. The zest of planning and performing experiments and interpreting their results, the continuous formulation, exchange, and discussion of new ideas, the intensely critical approach to our own work as well as that of others were among the key elements in an exceptional experience. Although our papers often did not have ready acceptance by reviewers and journals, still, recognition of the significance of our work was rapid. In 1954, Dr. Berson was proposed and accepted for membership in the Society for Clinical Investigation, no small achievement for someone without formal training in investigation or medical school affiliation. In 1957, he received the first of many honors to come, the first Eli Lilly Award of the American Diabetes Association. In 1960 we jointly shared the first William S. Middleton Award of the Veterans Administration. This was particularly meaningful because of the warm encouragement our efforts had always received from Dr. Middleton, first when he served as Chief Medical Director of the VA, and ever since.

Through the years we enjoyed the training of a limited number of Fellows in our laboratory. Among the best known of those who remained in investigation are Marcus Rothschild, Sheldon Rothenberg, Jesse Roth, and Seymour Glick. There would be alternate periods with and without Fellows for we chose not to have a laboratory any larger than we could manage with our own hands.

Our Radioisotope Service enjoyed the confidence and support of the Veterans Administration, locally and in Central Office; the laboratory was funded to the full extent of our requests without a need ever to submit a research grant proposal. We were completely free to develop in our own fashion. On occasion, when we had time to reflect, one of us would voice the thought, "How fortunate to be able to do only what one wants to—and to be paid for it!"

In 1967, the Bronx VA became associated with Mount Sinai School of Medicine and Dr. Berson was offered the Chairmanship of its Department of Medicine. For the first time he accepted another affiliation, although he did not relinquish the title of Senior Medical Investigator of the VA. This was the first offer which would not necessitate moving our laboratory. Sol hoped that by bringing to medical education the dynamic, imaginative approach which characterized his career in investigation, he could effect equally important advances in a new area.

In June 1967, on the last day he spent as a full-time investigator in our laboratory he expressed the hope that 17 years later he could again say that he had had the most wonderful of jobs. This was not to be. In his last letter before leaving London in January 1968 to take up his duties at Mount Sinai he wrote of his hope that his schedule would permit him to arrive at the VA by 4 P.M. at least twice a week to start "two full days of lab work." This also was not to be.

Yet he remained a complete partner in the laboratory until the end. His last act before leaving for Atlantic City was to come to his lab.

Sol Berson aspired to revolutionize medical education and, had he lived, he might have succeeded. Yet, perhaps he dreamed the impossible dream. In retrospect, one can wonder whether the times and the nature of medical institutions are as ready for that revolution as they were in the 50's and 60's for his explosive effects in medical investigation. We know the loss to medical investigation by Sol's devotion of his time and effort to his new role. We can but hope it will be compensated by his having set a model to which, in spite of these troubled times, a vibrant new medical school can aspire.

Meanwhile, the laboratory will remain in title as it always has been in spirit, "The Solomon A. Berson Research Laboratory," dedicated in his memory to his spirit of excellence and integrity.

# Solomon A. Berson and Rosalyn S. Yalow: A Scientific Appreciation

IRVING L. SCHWARTZ, M.D.

Sol Berson came to the Bronx Veterans Administration Hospital (now an affiliate of the Mount Sinai Medical Center) as a resident in July 1948. In April of 1950 he met Rosalyn Yalow, who had established a Radioisotope Unit at the hospital in 1948 and was seeking an internist to work in the new facility. Roz consulted Dr. Bernard Straus, Chief of the Medical Service who suggested that "the brightest fellow around here is Sol Berson who can be difficult, but it's worth putting up with any problems he may raise." Roz promptly sought out the candidate who spent most of the interview challenging her with math problems. "I was furious," Roz recalls, "but I did the problems. Later I realized he wasn't testing me—but simply that he thought, as a physicist, I'd enjoy them."

While contemplating returning to Bedford, Massachusetts, an army roost with fond memories, Sol was offered, accepted, and addressed himself to the job of Internist to the Radioisotope Unit (and to a transient episode of moonlighting in practice). Thus, the uniquely productive, mutually treasured Berson-Yalow scientific partnership was initiated. This partnership was to last throughout Sol's life. It was not long before their first research effort, a study of the use of $K^{42}$-tagged erythrocytes in blood volume determinations, was published (1). This paper initiated a series of precise studies (2–5) on blood volume (BV) which analyzed and placed in proper perspective discrepancies between BV as determined from plasma volume (using $T_{1824}$ or $I^{131}$-albumin) and BV as determined from red cell volume (using $P^{32}$-tagged erythrocytes). Having recognized the general muddiness of this field of investigation, they were the first to carry out a careful simultaneous study of this type that dealt with and evaluated the major problems in BV measurement and, thereby, made it possible to debug the prevailing methodology.

During this period they were involved also in clinical studies of the effects of cortisone in Hodgkin's disease (6, 7) and in the development of methods for the study of thyroid function (8, 9). Taking a cue from Myant, Pochin, and Goldie's determination of thyroidal iodide clearance by measuring uptake for two to four hours and blood levels on multiple blood samples taken during that period, Berson, Yalow, Sorrentino, and Roswit (8) showed that the iodide space amounted to 20 percent of body weight and, accordingly, that knowing the dose of labeled iodide injected, it was possible to determine the thyroidal clearance of iodide simply by measuring the iodide uptake of the thyroid gland. Furthermore, by comparing the thyroidal clearance and the renal clearance of iodide, they were able to explain the overlap in thyroidal iodide uptake of euthyroid

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and hyperthyroid subjects. Thus, they made it feasible to use the thyroid clearance as an important diagnostic test.

In continuation of the foregoing studies on thyroid function, Berson and Yalow (10) determined the thyroidal iodine secretion rate and the metabolic degradation and fecal excretion of organic iodine. In essence, by simply giving a tracer dose of radioiodine and then following the radioactivity of blood, urine, feces, and the thyroidal uptake before and after the administration of a blocking dose of an antithyroid drug, they were able to evaluate completely the quantitative aspects of iodine metabolism (10, 11).

A companion study (12) showed that normally the conversion of inorganic to organically bound iodine (organification) is extremely rapid but that after the administration of Tapazole® organification can be completely blocked, all uptake taking place simply as trapping of iodide. This paper had very important implications for the treatment of hyperthyroidism with antithyroid drugs such as Tapazole or propylthiouracil (PTU) because it demonstrated that, even though organification is virtually instantaneous, blockage of organification can be complete with adequate dosage and fractionating the dose at appropriate intervals prevents "escape" from the action of the antithyroid drug.

As an outgrowth of the earlier work on blood volume, Berson, Yalow, and their associates (13) studied the disappearance from blood of $I^{131}$-labeled human globin, then advocated as a plasma expander. They found that globin disappears so rapidly as to preclude its use as a plasma expander and, indeed, following publication of their study, the use of globin for this purpose was discontinued. As an interesting ancillary observation, they scanned the liver and noted that radioactivity was accumulating there, presumably reflecting uptake of globin by the reticuloendothelial cells.

In the course of the latter study, the disappearance of $I^{131}$-labeled globin was compared with the disappearance of $I^{131}$-labeled human serum albumin. In fact, the metabolic fate of the latter was a problem to which they devoted considerable effort for several years to come. In an elegant group of papers on the distribution and degradation of $I^{131}$-albumin (14–16), they drew attention for the first time to the fact that damage to a labeled serum protein was reflected in a shorter survival (half-life) in the circulation. They went on to relate the number of iodine atoms introduced per molecule of albumin to the shortened half-life and showed that with sufficiently "light" labeling (e.g., 1 iodine atom per molecule) and with protection against radiation damage, the $I^{131}$-albumin had a more prolonged survival in the circulation than the more highly labeled preparations which they and others had used previously. In fact the disappearance rate of their "lightly" labeled $I^{131}$-albumin preparation approximated the disappearance rate of natural albumin. It is noteworthy that one of these studies (14) involved the first application of a three-compartment model to the analysis of the equilibration and degradation of a labeled protein.

There followed a group of studies on blood volume and albumin turnover in congestive heart failure (17, 18). One of their research fellows who participated in this latter work, M. A. Rothschild, has continued the study of plasma protein metabolism in his laboratory and is today an outstanding contributor to this area.

With the techniques for working with labeled proteins more than mastered, the hyperacute Berson-Yalow curiosity became fixed on testing the possibility (suggested by Arthur Mirsky) that diabetics might have excessive insulinase activity and, accordingly, exhibit a rapid rate of insulin degradation. To test this hypothesis they administered $I^{131}$-labeled insulin intravenously to normal and diabetic subjects and, to their surprise, found a slower rather than faster disappearance rate. They pursued this interesting finding with a series of elegant experiments and incisive interpretations which culminated in a paper entitled "Insulin-$I^{131}$ Metabolism in Human Subjects: Demonstration of Insulin-Binding Globulin in the Circulation of Insulin-Treated Subjects" (19). This paper, which I had the privilege of reading in the form of an early draft, was rejected by two leading journals and its publication was held up for almost a year. However, after it finally was published, not many months were needed for it to be recognized as a masterpiece. Looking through the retrospectroscope it is now obvious that the findings reported in this paper presaged and provided the foundation for the magnificent achievements that were to come. To cite only two of its highlights: It demonstrated the universal antigenicity of insulin in man (later extended to other polypeptide hormones and other species); It contained a description of all the essential phenomena needed for the development of radioimmunoassay (and all other competitive binding assays). The manuscript had been rejected chiefly because some of the reviewers felt that the antibody nature of the insulin-binding substance had not been established, although its properties were consistent with the criteria for antibody as defined by such authorities as Topley and Wilson. However, there were other problems. The idea of a soluble antigen-antibody complex was not generally accepted at the time nor was an equilibrium (affinity) constant of $10^{10}$ L/M for the insulin-insulin antibody reaction easily swallowed by the immunochemists who believed, based on Pauling's theoretical analysis, that the equilibrium constant for antigen-antibody reactions was in the range of $10^{6}$ L/M. In addition there was at the time a deep-seated prejudice that peptide hormones were nonantigenic. In one of the letters of rejection, a reviewer wrote "Everybody knows that insulin is generally not an antigen. After all we've used insulin therapeutically for 30 years. If it were significantly antigenic, we would have known about this long ago."

As noted above, the phenomena which form the basis for radioimmunoassay and all other types of competitive binding assay were clearly revealed in this landmark paper which finally appeared in the *Journal of Clinical Investigation* in 1956, but three years more were to pass before Berson and Yalow reported on the measurement of the level of immunoreactive insulin in human plasma. However the term "radioimmunoassay" was coined much sooner—in the last paragraph of an abstract (20) submitted to the 1957 Annual Meeting of the American Society for Clinical Investigation.

During the period 1956–1961 Sol and Roz worked extensively on the problem of radiation damage to labeled proteins and peptides (16, 21–25) and on the kinetics of the reaction between insulin and its antibody and on other parameters of the system (26–31). In 1959, they published a definitive paper (30) on the quantitation of the reaction between antigen and antibody in which they established the conditions necessary for the development of *any* immunoassay for *any* antigen. This was the first satisfactory treatment of the system involving labeled antigen and soluble antigen-antibody complex. A companion paper (31) demonstrated that human insulin reacted weakly with human antibodies against beef-pork insulin. Thus it became clear that human antisera could not be used for assay of human insulin and that appropriate animal antisera (e.g., guinea pig antisera) would be needed.

The stage was now set for the first announcement of the assay of plasma insulin in human subjects (32) which was followed by a definitive publication (33) on this achievement within a year. This latter paper is more than an account of the immunoassay of insulin in man—it is a virtual handbook of principles and procedures for validation of any immunoassay. In addition, this paper carried a major contribution to our knowledge of human diabetes, namely, the discovery that maturity-onset diabetics had a delay in the secretion of insulin in response to hyperglycemia but that their time-integrated output of insulin was greater than that of normal subjects. Shortly thereafter a number of related papers appeared (34–36) and further attention was given to various aspects of the insulin assay (37–40). In 1961 they observed that insulins purported to have identical amino acid sequences, although from different species (whale, dog, pig), cross-reacted differently with some human antisera, suggesting that primary sequence does not necessarily determine the preferred conformation of the molecule (41). They allowed for the possibility that some molecular alteration(s) may have occurred during the isolation of the hormone but, in view of the fact that it is now known that there are differences in the proinsulins of dog and pig, the intriguing possibility remains that during biosynthesis each C-peptide confers a conformation upon its associated insulin which is retained after the latter is cleaved from the prohormone.

After reviewing the foregoing work on plasma insulin in health and disease (42) and the nature and treatment of insulin resistance (43), Sol and Roz proceeded to address their methodology to physiologic and pathophysiologic problems involving parathyroid hormone (44), growth hormone (45–48), and ACTH (49, 50). The development of an immunoassay for bovine and human parathyroid hormone (PTH) was carried out in collaboration with Gerald Aurbach and John Potts, but the physiological and clinical application of the method was to come later. The work on growth hormone was carried out in collaboration with Seymour Glick and Jesse Roth who spent two years as research fellows in their laboratory during 1962 and 1963. Prior to their work it was, of course, well known that growth hormone secretion was elevated in acromegaly and that excesses led to diabetes, but the physiologic role of growth hormone in the normal adult was completely unknown. Their papers delineating this role revealed that growth hormone secretion was stimulated by intracellular deprivation of glucose and by stress (45–48); other investigators soon showed that amino acids and protein are also growth hormone secretagogues.

In 1964 Sol and Roz reported on the radioimmunoassay of corticotrophin (ACTH). In their initial paper on the methodology (49) they showed the effect of stress on the plasma level of immunoreactive ACTH and also the phenomenon of negative feedback (fall in the plasma ACTH level following the administration of cortisone). The complete methodologic and physiologic study of ACTH immunoassay was published four years later (50) and included a demonstration of the diurnal rhythm of the plasma ACTH level as well as additional data on stress and feedback (response of the plasma ACTH level to hypoglycemia and dexamethasone, respectively).

While the work on PTH, growth hormone and ACTH was under way, they continued their studies on insulin in relation to diabetes (51), obesity (52), and hypoglycemic states (53), and they further refined the methodology for immunoassay in general (54–56) and PTH in particular (57). Shortly thereafter they published the first demonstration (58) of elevated plasma PTH levels in hyperparathyroidism, bronchogenic carcinoma, and chronic hypocalcemia associated with uremia. At the same time they participated in a demonstration (59) of the development of clinical resistance and antibodies to PTH employing $I^{131}$-labeled PTH in a reversal of the usual use of immunoassay. In the following year they found that immunoreactive PTH in plasma differed from PTH extracted from the gland and also that the difference in the PTH from these two sources was more marked in patients with uremia than in patients with primary hyperparathyroidism (60), discoveries resulting from their preparation and use of two different classes of antisera that were capable of recognizing the immunochemical distinctions. Thus, this work revealed that there were at least two different types of PTH in a single individual.

Several of their projects in the mid-sixties clarified various aspects of glucose tolerance, insulin secretion and the etiology of diabetes. For example, they demonstrated that the insulin insensitivity associated with maturity-onset diabetes (52) is independent of the insulin insensitivity induced by obesity although when both conditions coexisted, the combined insensitivities result in higher serum insulin levels than in either condition alone. Thus, in those diabetic patients with adequate pancreatic reserves, the hypersecretion of insulin modifies the hyperglycemia; however, in patients with increasingly inadequate pancreatic reserves, the plasma insulin levels are progressively lower and the hyperglycemia not only persists but becomes progressively more severe (52). Another striking aspect of this study was the observation that the modest fall in blood glucose late in a glucose tolerance test leads to a substantial increase in the secretion of growth hormone (52). Knowing that growth hormone has an insulin-like action (which "improves" glucose tolerance) in the first hour or two after administration but thereafter that this hormone reduces glucose tolerance, they went on to show that the endogenous release of growth hormone late in a glucose tolerance test affects the insulin sensitivity during a second glucose tolerance test carried out directly or a few hours after the initial test (53).

These latter findings provided an explanation for the "afternoon diabetes" of individuals who are found to have diminished glucose tolerance in a test conducted in the afternoon (6 hours after breakfast). It was also shown that a) the secretion of growth hormone did not parallel the secretion of ACTH in response to stressful stimuli (62); b) diabetic patients secrete insulin sluggishly in response to a glucose challenge but normally in response to a tolbutamide challenge (63); and c) since glucose- and glucagon-stimulated insulin secretion is blocked by prior administration of 2-deoxy-d-glucose but tolbutamide- and arginine-stimulated insulin secretion is not, the mechanism involved in the action of these two groups of secretagogues must differ (63).

Following Steiner's identification of proinsulin, Jesse Roth and associates at the N.I.H. demonstrated that this prohormone ("big insulin") was present in plasma and it was suggested that the presence of immunologically active but biologically inactive insulin might account for the hyperinsulinism of diabetes. However Sol and Roz showed that "big insulin" was only a minor plasma constituent in diabetes and other states characterized by insulin insensitivity (64, 65), although it was the major immunoreactive form of insulin in some cases of insulinoma (65). Most recently they have identified in the plasma of an insulinoma suspect a new hormonal form designated as "big, big insulin" (66). This new species of prohormone, the primary form of immunoreactive insulin in this patient, eluted in the void volume of a Sephadex G50 column. It was further characterized on Sephadex G100 and G200 columns, on starch gel and starch block electrophoresis and by the preservation of its immunochemical identity following tryptic conversion to insulin (i.e., no change was observed in the total immunoreactivity after conversion by trypsin treatment; thus, their antibody "sees" the same antigenic site whether this site is presented on insulin, proinsulin or "big, big insulin"). It may well be that this is the ultimate precursor for the insulin family.

In 1970, their now classic immunoassay methodology was applied to the measurement of Australian antigen (67, 68), a viral antigen implicated in hepatitis. The method, developed together with J. H. Walsh, provided a 100- to 1000-fold increase in sensitivity over the available conventional methods for detecting this antigen (67 68).

In the late sixties they became interested in the gastrointestinal hormones and, particularly, in gastrin. Following McGuigan's initial reports on radioimmunoassay of gastrin in plasma, assays were developed in their laboratory (69) and elsewhere. They found that the hypergastrinemia, secondary to the hypoacidity of pernicious anemia, was suppressible by the administration of HCl, thus demonstrating once again the significance of hormone-substrate interrelationships. Thereafter their work on gastrin followed two different paths, one concerned with the molecular nature of gastrin in the plasma and the gastrointestinal tract, the other concerned with the clinical and physiologic significance of the hormone. Moving on the latter path, they found that the effect of atropine on parietal cells (suppression of HCl secretion) is associated with an increase in gastrin secretion in response to feeding presumably because of removal of the acid feedback mechanism which normally operates to inhibit gastrin release (70). They further demonstrated (71) that the gastrin response to a mixed feeding (orange juice, eggs, toast) was greater than to a pure protein or pure carbohydrate (glucose) feeding. They then showed that patients with the Zollinger-Ellison syndrome (gastrin-producing tumor of the pancreatic delta cells) did not respond to a mixed feeding with increased gastrin secretion (71). In addition, they identified a new class of ulcer patients characterized by marked hyperacidity, by marked elevations in plasma gastrin levels in the fasting state, and by feeding-stimulated gastrin levels which were well within the range for Zollinger-Ellison patients (71).

In studies of the chemical nature of gastrin they discovered that the major form of immunoreactive gastrin in human plasma differed from the heptadecapeptide gastrin (HG) extracted from the antrum and purified by Gregory and Tracy. On Sephadex gel filtration, the gastrin from human plasma eluted earlier than HG, indicating its larger size (72) and, accordingly, they referred to it simply as "big gastrin" or BG. In studying the electrophoretic mobility of gastrins in starch gel and paper systems, they found that BG was more basic than HG (72) and, on tryptic digestion, they found that BG was converted to HG with no loss of immunoreactivity (73). They demonstrated that: a) like insulin, gastrin also exhibited size and charge heterogeneity; b) the configuration of the immunoreactive site in "little" gastrin (HG) is the same as in "big" gastrin (BG); and c) as expected, HG, lacking arginine and lysine residues, does not break down further on continued treatment with trypsin. As a final *coup de grace* for this study they showed that both BG and HG are stimulated by feeding and that HG turned over (disappeared from the circulation) more rapidly (73).

In a companion paper (74) they demonstrated that although there are approximately equal amounts of antral and duodenal gastrin in humans, the antral hormone is primarily "little" and the duodenal hormone is primarily "big"; in fact, the ratio "big"/"little" increases with descent along the gastrointestinal tract. Thus jejunal gastrin is virtually entirely "big". Very recently, perhaps as an interim finale to the gastrin saga, they noted that a small fraction (one to two percent) of the total immunoreactive gastrin in the plasma of Zollinger-Ellison patients appeared in the void volume of a Sephadex G50 column (75). They dubbed this fraction "big, big gastrin" or BBG. In following up on this new form of the hormone, they found (a) that, like BG, BBG was converted to HG by trypsin and (b) that there was very little BBG in antral and duodenal tissue, but that in jejunal tissue BBG amounted to 25 percent of the total hormone content (75). This latter finding provided strong support for their concept that the larger precursor forms of the hormone become proportionately more abundant with descent in the gastrointestinal tract.

Stimulated by their encounters with the "big" and "big, big" forms of insulin and gastrin, they sought and found a component of immunoreactive ACTH which also elutes in the void volume of a Sephadex G50 column (76). This fraction, tentatively referred to as "big ACTH", has been demonstrated in extracts of anterior pituitary tissue, cancer tissue (specifically, a thymoma responsible for an ectopic Cushing syndrome), and in plasma where the "big ACTH" component may range from 0 percent (in Addison's disease) to 100 percent (conditions of ectopic ACTH production) (76).

I would like now to find the words to capture the essence of the foregoing summary of highlights of a profound and powerfully productive research career —developed within the framework of a magnificently synergistic scientific partnership. But how does one summarize a summary? Perhaps this has been done best already by the following statements of Neal Bricker and Sidney Ingbar, respectively:

"In collaboration with Dr. Rosalyn Yalow, Dr. Berson made contributions to our knowledge of such a fundamental nature as to transform and revolutionize a whole segment of clinical investigation. The tools developed by Berson and Yalow have become all-pervasive and their application has permitted not only the measurement of a host of small peptide hormones, but in recent years the measurement of an increasing number of diverse substances . . . Science and medicine have lost a great and distinguished colleague and for many the loss includes that of a close and personal friend."

"The true measure of Solomon Berson's greatness is not that he is deeply mourned by his many friends, not that he has been widely acclaimed and will be universally missed in the world of science, but that the lives of countless others who neither knew him nor knew of him have been touched and bettered by the knowledge that has derived from his creative genius."

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# Solomon A. Berson: The Sinai Years

IRVING L. SCHWARTZ, M.D., HORACE L. HODES, M.D.,
AND LOUIS J. SOFFER, M.D.

A 1938 graduate of the City College, a senior college in The City University of New York (CUNY) system, Sol Berson was to return to CUNY thirty years later as Professor and Chairman of the Department of Medicine of Mount Sinai School of Medicine and the Director of Medicine of The Mount Sinai Hospital. Sol's decision in 1967 to come to Sinai and take on the diverse and pressing responsibilities of Chief of Medicine was not an easy one. He was at the time a Senior Medical Investigator at the Bronx Veterans Administration Hospital where his days were filled largely with satisfying, intensive scientific and scholarly activity, and where the administrative demand on him was minimal. Nonetheless, he felt impelled to meet a new kind of challenge and to seek the more extensive personal and scholarly interactions with students, house staff, and faculty that the newly founded medical school could provide—and after his decision was made, he never regretted stepping into this new and very different mix of scholarly and executive activity, even when administrative problems were crushing and frustrations were maximal. He often spoke of the great satisfaction that he drew from his many and varied interactions with students and colleagues, and, reciprocally, they experienced a progressively deepening admiration and affection for him and, perhaps of greater importance, they found in him a limitless source of inspiration, all of which operated in some mode of positive feedback to enhance, subtly but profoundly, the communication between teacher and disciples. Some sense of Sol's impact on his students is conveyed by the remarks of Steven Port, a member of the Class of 1972 of the Mouth Sinai School of Medicine and now a house officer of The Mount Sinai Hospital:

"My first contact with Dr. Berson was as a second-year student here at Mount Sinai. It was the first lecture I had ever heard on the thyroid. I recall sitting for about an hour quite dumbfounded as an energetic, enthusiastic, incredibly knowledgable man gave the best lecture I had ever heard. I had heard rumors about Dr. Berson, but after that lecture I knew that I had been exposed to the greatest intellect of my educational career.

"It was not until I began my clerkship in medicine that I actually had any personal contact with him. It was the beginning of a very short association which I shall never forget. Not only was he eager and willing to discuss any medical or physiological or biochemical topic with any student for any length of time, but also he would talk of nonscientific topics, freely and with authority. It was always a pleasure to listen to him, even when we disagreed.

"We spoke often of the School, and the Department of Medicine, and the Hospital. And he openly shared his views on these topics with his students. And

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we became intimately involved with the activities of the Department of Medicine.

"Curiously, I only know his scientific achievements from a distance. They will perhaps be his most lasting claim to recognition. As was recently pointed out in an editorial in the *New England Journal of Medicine,* the accomplishments of Drs. Berson and Yalow have revolutionized an entire phase of laboratory medicine and have affected some of the fundamental principles of medicine. Although a controversial chief of medicine in some ways, there was nothing controversial about Dr. Berson's relationship with his students. He always had time for students. No favor was too great, whether it was helping on a case or calling all over the country to get someone an internship or an N.I.H. position. When he made his weekly staff rounds, we stood on tables in a packed conference room. And when he made rounds with medical students, the house staff would seek out the students for his opinion. He was able to discourse at length in almost any area of clinical medicine, physiology or biochemistry. For those of us who were fortunate enough to have been closer to him than a reference in a journal article or a few pages of lecture notes on peptide hormones, he was much more than the discoverer of radioimmunoassay. He was a model, an ideal, an inspiration. We all shared his vision of an academically incomparable Department of Medicine."

Sol's impact on graduate students was no less than that on medical students and house staff even though his lectures in graduate courses were usually scheduled for a two-hour period starting at 7:00 a.m. Five out of twenty-two graduate students showed up for the first of his lectures on Endocrine Physiology. But five were enough—the word got around—and the room was overflowing for his second 7:00 a.m. lecture, not only with the full complement of students who were registered for the course but with another thirty graduate student auditors who came from distant campuses such as Queens, Brooklyn, Hunter, and City College.

The rapport which Sol found with senior members of his faculty differed in some respect from that which he enjoyed with students, but it had a special quality and depth of its own—and his impact on his peers was equally powerful. This impact is exemplified in the following words of one of this latter group:

"What does one say about a man like Sol Berson? A few months ago, there was a meeting of the International Congress of Endocrinology in Washington, and a memorial for Sol was held at this meeting. The speakers were his friends, his colleagues and students from abroad and from this country. The hall was filled with scientists and physicians from all over the world, and the speakers were sincere and eloquent. The audience was involved and responsive, and altogether it was a very moving memorial. And yet when it was over, few felt comforted.

"I suppose there are times when words are very inept instruments to express the turmoil and grief which the death of such a man engenders. Which brings me back to the original question, what does one say about a man like Sol? Well, I would say that his death was a great tragedy, to his family and colleagues and his friends, to the institutions which he served and graced, and to the society in which he lived.

"He was a unique scientist. The work which he and Dr. Yalow, his colleague of so many years, have done has literally revolutionized the field of endocrinology and has had a progressively greater and greater impact on all of medicine and all of physiology. They won a good many prestigious awards to which they were, of course, justly entitled.

"But Sol was more than a great scientist, a superb musicologist, a mathematician, and chess player of note. He was a physician in the noblest sense. He had concern and compassion. The patient in bed was no mere cipher to him, but a person, anxious, frightened, who required thoughtfulness and sensitivity, qualities which Sol had in great abundance. He shared these qualities generously with all of mankind, particularly the underprivileged and the disadvantaged. He was a man of great conviction and integrity. He had no commerce with cant, hypocrisy, and glibness. He had a strong sense of right and wrong, and an unyielding courage to fight for what he thought was right. It was no accident that he had so many friends and admirers, and, by the same token, these very qualities outraged other, I presume lesser, men.

"This was Sol Berson. He was a wonderful teacher, a prodigious intellect, with a vast knowledge of medicine—almost all fields of medicine. Perhaps he overwhelmed the students and his colleagues, but he prodded and he stirred and he excited and he challenged the mind and the imagination. He expanded the vision and the horizons of thinking of all men and women who were prepared to listen to him.

"This was Sol Berson; he was this kind of a man. Never a complacent person himself, he would have no tolerance for complacency in others. He knew that in the quest to become a great physician, a great scientist, or a great institution, complacency was a fatal defect. To achieve this kind of greatness required a restlessness of spirit, a mind in ferment, constant questioning, insatiable curiosity and ceaseless effort. These qualities he brought to our institution.

"It is said that no man is indispensable. I'm not sure that this is true and indeed I think it isn't. There are men and women who discover highways and byways that were previously dark and therefore unknown and, once having illuminated them, they remain bright forever. This precious group, this small impressive group of people of whom Sol was one, is in the largest sense indeed indispensable.

"The untimely death of Sol and his daughter, Wendy, was a paralyzing horror to all of us. And we grieve with his family. We grieve very deeply. We grieve also for our institution, because his death has left a void which will not be readily filled. And as for myself, I lost a friend, an inspired and inspiring friend, whose like I shall not encounter again."

These words of friend and student speak for the richest aspects of Sol's years at Sinai. They enlarge our already vast sense of loss, but at the same time they remind us that our association with Sol has left us with something that can never be lost—the legacy of his achievements, aspirations, personal and scholarly standards, and the model of his integrity and essential humanity.

# Radioimmunoassay of Human Plasma ACTH and the Pathogenesis of Cushing's Disease*

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The Berson-Yalow radioimmunoassay method for the measurement of human plasma ACTH has all but settled the question whether pituitary ACTH is or is not secreted excessively in untreated Cushing's disease (1). Earlier studies, carried out by a variety of biologic assay techniques, had provided strong suggestive evidence of excessive ACTH secretion in the disorder, but it was not until the data provided by radioimmunoassay were published that the matter was placed on a solid, quantitative basis (2). The arduous developmental work that yielded a feasible immunoassay for ACTH has thus succeeded in defining more clearly a subtle biologic abnormality.

From Cushing's description of pituitary basophilic adenomas (1932) and P. E. Smith's demonstration that pituitary extracts exert tropic actions on the adrenal (1930), it seemed plausible to suppose that hyperadrenocorticism not associated with adrenal tumor had its origin in some abnormal function of the pituitary. The history of the various "hypothalamic," "pituitary," and "primary adrenal" theories of the pathogenesis is reviewed elsewhere (3); by the mid-1950's most people had begun to believe that there was, indeed, excessive pituitary ACTH secretion, but the evidence was circumstantial (4, 5). The period 1955–1962 brought forth further circumstantial evidence through bioassay techniques, but, as will be shown, there was no satisfactory proof of ACTH hypersecretion in Cushing's disease until 1964 and later, that is, until the era of ACTH radioimmunoassay.

As early as the 1930's there had been a few attempts to discover increased ACTH concentrations in the plasma of patients with Cushing's disease. Taylor, Albert, and Sprague cite four reports in the period 1935–1942 showing adrenal weight-maintaining activity in plasma of "Cushing's syndrome" (6). Lack of sufficient controls and manipulations of the serum likely to destroy ACTH (deproteinization, heating) throw doubts on the specificity of those findings. With the Sayers assay (adrenal ascorbic acid depletion) Taylor et al were themselves unable to observe "adrenotrophic activity" in serum of patients with Cushing's disease, but could detect it in serum of addisonians (6). By more sensitive bioassay methods, most workers have also found ACTH more easily in addisonian plasma than in that of Cushing's disease (see, for example [7]). Getting ahead of the narrative, it has turned out that plasma ACTH concentration is indeed higher in untreated Addison's than in Cushing's disease, a matter only clarified by ACTH immunoassay (2).

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* The term 'Cushing's Disease' is used in this paper to mean the form of Cushing's syndrome (hypercortisolism) associated with bilateral adrenal cortical hyperfunction presumably owing to excessive anterior pituitary secretion of ACTH.

With the use of the Simpson-Evans-Li bioassay for ACTH (partial prevention of adrenal atrophy), Jailer et al were able in 1957 to detect quite consistently the presence of adrenal weight-maintaining activity in plasma of patients not yet adrenalectomized for Cushing's disease (8). Initially, Jailer et al failed to demonstrate this activity in Addison's disease but did find it in later attempts (9). These and further studies (10) established that the activity found in Cushing's plasma was dependent upon ACTH activity and upon a plasma potentiating factor (11) whose nature is still not clear. The assay used, though cumbersome and relatively insensitive (8), disclosed adrenocorticotropic activity in 17 of 21 patients with Cushing's disease, a frequency far higher than that achieved by other biological assays before or since (10). However, the great disadvantage was that the assay did not permit quantification of the ACTH or ACTH-like activity in plasma.

With various modifications of the Lipscomb-Nelson bioassay (increased adrenal secretion of corticosterone), it was usually not possible to find elevated ACTH concentrations in untreated Cushing's disease. Presumably, the concentrations were below the detectable range, so Williams et al studied bilaterally adrenalectomized Cushing's patients who were found to have higher plasma ACTH levels than addisonians treated with cortisol and having equal plasma cortisol values (12). These data were later used to sustain a hypothesis that the pituitary is "driven," perhaps by the hypothalamus, to oversecrete ACTH in Cushing's disease (7), and evidence was cited to show that elevated plasma ACTH values were not a necessary condition for the pathogenesis of the disease, since administration to normal people of extremely small quantities of ACTH could be shown to produce some of the physiologic and biochemical features (5, 7). Later work still showed absence of raised plasma ACTH concentration in the untreated disease: again with the Lipscomb-Nelson assay, Ney et al found levels above normal in only three of nine patients (13), and Nelson, Sprunt, and Mims reported only three of 13 values significantly above normal in untreated subjects (14).

Up to this point (1963–1966), the evidence for excessive ACTH secretion in untreated Cushing's disease was thin. The earliest evidence gathered with immunologic techniques was not definitive, either. In their first paper, Yalow and Berson and their coworkers reported high plasma ACTH values in Cushing's syndrome, but only in patients with extrapituitary, "ACTH-producing" tumors or in patients with Cushing's disease *after* adrenalectomy (1). With a modified Yalow-Berson (1) technique, Demura et al found raised values of plasma ACTH in only one of two patients (15). By means of a similar but homologous immunoassay (using human instead of porcine ACTH for immunization) Landon and Greenwood did better; they found moderate elevations of plasma ACTH in four of five untreated patients (16); this series was soon expanded to include 20 patients, in ten of whom plasma values were raised (17). Further, patients with Cushing's syndrome and adrenal tumor had values of ACTH below normal, and, as others had shown (1, 12), values were greatly elevated after bilateral adrenalectomy for Cushing's disease (17). In their own

TABLE I

*Some Estimates by Bioassay and Radioimmunoassay of Plasma ACTH in Untreated Cushing's Disease*

| Authors | Method | Values in Patients | Normal Values | No. of Abnormal Values/No. of Patients |
|---|---|---|---|---|
| Taylor et al (6) | Sayers | 0* | 0 | 0/2 |
| Jailer et al (8) | Simpson-Evans-Li | Detectable | 0 | 8/9 |
| Segal et al (10) | Simpson-Evans-Li | Detectable | 0 | 17/21 |
| Ney et al (13) | Lipscomb-Nelson | 0.14–1.81 mU/100 ml | 0.10–0.59 mU/100 ml | 3/9 |
| Nelson et al (14) | Lipscomb-Nelson | >1.00 mU/100 ml | <1.00 mU/100 ml | 3/13 |
| Demura et al (15) | Yalow-Berson (modified) | 0.6,3.9 mU/100 ml | 0.30–0.70 mU/100 ml | 1/2 |
| Landon et al (16) | Yalow-Berson (modified) | 50–125 $\mu\mu$g/ml | 12–55 $\mu\mu$g/ml | 4/5 |
| Besser et al (17) | Yalow-Berson (modified) | 40–200 $\mu\mu$g/ml | 12–60 $\mu\mu$g/ml | 10/20 |
| Berson et al (2) | Yalow-Berson | 200 $\mu\mu$g/ml | 0–75 $\mu\mu$g/ml | 1/1 |

* 0 denotes "not detectable".

exhaustive survey, Berson and Yalow were able to study only one untreated patient, whose plasma value was definitely raised (2) (Table I).

There are still important unanswered questions about the possibility that different materials are being measured by immunoassay versus bioassay (18), about the nature and characteristics of other corticotropins that have been reported in the pituitary (19) and in the plasma of patients with Cushing's disease (8, 10, 11), and about the true significance of alleged abnormalities in "diurnal variation" of cortisol and ACTH secretion (20). But it seems clear that the Berson-Yalow radioimmunoassay has established quite definitely an excess of ACTH in plasma of most patients with untreated Cushing's disease (1, 2, 15–17). The data have shown that the elevations of ACTH are often slight, so that only a highly accurate and sensitive method would serve to detect them. Berson and Yalow devised such a method and had only just begun to apply it (1, 2).

The theoretical contribution and the innumerable practical uses of the radioimmunoassay technique have been often praised. But for the brilliance of Berson's imaginative stroke, for the dogged work needed in its application, and for all the new things it has made possible, no praise is too high*.

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* The writer had several hours alone with Berson only once. It was about a year before his death, during an auto trip from Atlantic City back home to New York in April, 1970, after we had both taken part in an AMA meeting. The talk rambled over everything: teaching medicine, managing a medical service, European history, books, child-rearing, souvenir-buying, music. Arriving in the city, we stopped at a bookstore on the corner of 12th Street and Broadway, a favorite haunt of Berson's. He dashed around among the piles of books, unearthing 18th- and 19th-century historical works I had never heard of. After we, or rather he, had exhausted the possibilities of the place, we ran out through the rain to the car. About then, it suddenly occurred to me that I had never met anyone like him, anyone of equal capacity. He was qualitatively different.

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# Neurotransmitter Regulation of ACTH Release

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It is generally accepted that many aspects of ACTH release are modulated by the central nervous system. Such regulation is mediated by a corticotropin-releasing factor (CRF) (and possibly a corticotropin-inhibitory factor). The site of production of such a factor(s) within the central nervous system, and its chemical structure, still remain to be elucidated. It is accepted that such production occurs within neurosecretory cells which, in addition to having secretory properties, share with other nerve cells the property of synaptic activation by neurotransmitter substances.

Although the identity of the neurotransmitters which effect synaptic transmission within the central nervous system has not been rigorously established, it is presumed that some of these transmitters are similar to those whose function has been described in the peripheral nervous system. Chemical and fluorescence techniques have demonstrated the presence of acetylcholine, norepinephrine, serotonin, and dopamine within the central nervous system, as well as the presence of enzymes involved in their synthesis and degradation. More specifically, regional and circadian variation of levels of these substances within areas of the hypothalamic-limbic system has been demonstrated. These are areas which have been shown, through lesion and stimulation experiments, to have a marked effect on ACTH secretion (1) as well as to be sites of corticosteroid concentration (2).

It would appear likely, therefore, that these transmitter substances, by acting on the neurosecretory cells involved in the production of CRF, could effect its release or inhibition in response to the varied stimuli which are known to affect ACTH levels. There is no evidence (3) that such neurotransmitter substances may effect ACTH release by a direct action on the pituitary gland.

Investigations concerned with the role of neurotransmitter substances in the regulation of CRF release have utilized diverse approaches. These have included assessing the effects on various aspects of pituitary-adrenal function of systemic, intra- and extrahypothalmic or intraventricular administration of neurotransmitter substances or drugs which block their action or synthesis, as well as the effects of lesions or chemical substances which destroy specific neurotransmitter pathways. As may be seen in the accompanying tables, investigations have at times led to contradictory results. The present paper will attempt to explore some of the methodologic problems which may lead to such inconsistencies, and summarize the general conclusions that may be drawn at present with regard to neurotransmitter regulation of ACTH release. Although the role of ACTH and corticosteroid levels in modifying neurotransmitter effect on CRF is an important one, consideration of this aspect is outside the scope of this review.

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## *Problems of Methodology in Assessing Neurotransmitter Regulation of ACTH Release*

1. *Role of state of consciousness and stress:*

In evaluating the effects of neurotransmitter agents on basal ACTH or cortisol levels, the use of unrestrained, unstressed, conscious animals is desirable. There is evidence that a given chemical or electrical stimulus may have opposite effects on pituitary adrenal function if the stimulus is given at a time of high (as seen in stress) or low (basal) plasma corticosteroid levels. There is also evidence that anesthesia may modify the effect of a given neurotransmitter substance. It has been noted (4) that whereas serotonin exerts an inhibitory effect on hippocampal unit cell recording in the barbiturate-anesthetized animal, a stimulatory effect is seen when unanesthetized decerebrate preparations are studied.

2. *Site of drug administration:*

(a) *Intracerebral vs intraventricular.* A difference in pituitary-adrenal responsiveness might be expected if a transmitter substance or blocking agent is injected intraventricularly rather than into a specific area of the central nervous system. It has been demonstrated (5, 6) that implantation of a given neurotransmitter may lead to inhibition or stimulation of pituitary-adrenal function depending upon the central nervous system area into which it is implanted. Intraventricular administration may lead to widespread transport of the substance being administered to both stimulatory and inhibitory areas, the exact response being dependent upon the relative proximity to the ventricular system of such areas. A similar objection may pertain to intraventricular injection of either 6-hydroxydopamine or 5,6-hydroxytryptamine, which act by selectively destroying noradrenergic (7) and serototoninergic (8) neurons respectively.

(b) *Systemic vs. intracerebral.*

(1) Systemic administration of drugs which modify central neurotransmitter synthesis, metabolism or action would also be expected to affect equally all intracerebral (stimulatory and inhibitory) loci at which the neurotransmitter might exert a stimulatory or inhibitory effect.

(2) Systemic administration of drugs which modify neurotransmitter synthesis, metabolism or action would be expected to affect peripheral as well as central neurotransmitters. It might be then unclear as to whether hormonal changes observed were secondary to direct effects of these drugs on central neurotransmitters, or indirect ones secondary to changes in central neurotransmitters brought about by peripheral changes in blood pressure, intravascular volume, etc.

3. *Assay of neurotransmitter content:*

(a) *Regional variation.* It has already been noted that there is regional variation of specific neurotransmitters. Therefore, studies in which only whole brain levels of such transmitters are determined might mask more specific changes in the loci actually involved in CRF regulation.

(b) *Circadian variation.* Since there is a circadian variation in neurotransmitter content, studies should specify the time of day at which this is determined.

(c) *Importance of measuring several neurotransmitters simultaneously.*

(1) *Drug effects on more than one neurotransmitter:* It has been assumed that agents will affect specifically concentrations of the given neurotransmitter in whose metabolic pathway they have been shown to act. For example, the steps in serotonin synthesis are depicted as:

$$\text{tryptophan} \xrightarrow[\left(\substack{\text{blocked by}\\ \text{PCPA}}\right)]{\left(\substack{\text{tryptophan}\\ \text{hydroxylase}}\right)} \text{5-OH tryptophan} \xrightarrow{\text{(decarboxylase)}} \underset{\text{(serotonin)}}{\text{5 hydroxytryptamine}}$$

However, tryptophan loading in dogs has been shown to increase homovanillic acid (HVA) levels in cerebrospinal fluid (9) indicating an effect on dopamine (since HVA is the main acid metabolite of dopamine). Administration of 5-OH-tryptophan lowers brain norepinephrine levels (10), perhaps by competing for the same decarboxylase:

$$\text{tyrosine} \xrightarrow[\left(\substack{\text{blocked by}\\ \text{aMPT}}\right)]{\text{(hydroxylase)}} \text{DOPA} \xrightarrow{\text{(decarboxylase)}} \text{dopamine} \longrightarrow \text{norepinephrine}$$

Therefore, unless concentrations of both transmitter substances are measured, it would be inaccurate to ascribe any observed effects to changes in just the one transmitter studied.

Certain agents employed, (i.e., reserpine, monoamine oxidase inhibitors) will affect levels of both serotonin and norepinephrine, yet hormonal effects observed may be erroneously ascribed to alteration in just one of these substances.

(2) *Importance of neurotransmitter ratios.* There is evidence (11) that it is the ratio between two neurotransmitter substances rather than the actual levels of a given neurotransmitter that is important in effecting CRF release. This is a further reason for measuring levels of several neurotransmitters when studying the effect of a given pharmacological agent.

4. *Time of drug administration:*

In addition to the importance of measuring levels of transmitter substances at a constant time of day (because of circadian variation) this is also an important parameter in evaluating neurotransmitter effect. It has been noted that direct application of norepinephrine to the hypothalamus in the dark (a period of elevated hypothalamic norepinephrine levels) suppresses feeding behavior, whereas increased feeding is noted following such application during the light period (12). It is to be expected that other parameters, including hormonal ones, will vary similarly.

5. *Concomitant rather than causal changes:*

Certain experimental interpretations have assumed cause and effect in the interrelationship of two variables, whereas such a relationship may merely reflect concomitant changes in these variables in response to another factor. For example, the fall in brain norepinephrine levels which occurs in some "stress" situations, concomitant with elevation of corticosteroid levels has been interpreted as indicating adrenergic inhibition of pituitary-adrenal function. Not only can such parameters be changing independently of each other in response to the "stress" but it has been demonstrated that in such "stress" situations norepinephrine turnover is increased (13) (as is that of serotonin (14)) so that it would be proper, as has been done, to invoke adrenergic stimulation of pituitary-adrenal function in such situations (15).

6. *Site of stimulation may not be site of CRF production:*

In studies involving direct intracerebral implantation of neurotransmitter, where many of the above considerations do not apply, it is important to note that observing an hormonal effect of neurotransmitter at a given site does not necessarily imply that that site is directly involved in CRF synthesis. Instead the site may be a link in a multisynaptic pathway leading to "the final common pathway" (16) for CRF release. For example, it has been shown that a septal lesion will lower serotonin concentration only in the hippocampus, of the numerous CNS areas so studied (17). Septal stimulation by a given neurotransmitter may likewise effect a hippocampal locus, which by itself may be responsive to the same or a different neurotransmitter.

7. *Dose of substance employed:*

The dose of neurotransmitter employed should be within the physiologic range for the tissue being studied. As rough approximations, hypothalamic concentrations (at the time of the circadian peak) of serotonin, norepinephrine, and acetylcholine in the rat are 1.0, 1.9, and 2.9 $\mu$g/gm respectively, with rat hypothalami weighing approximately 150 mg. Even allowing for differences in solubility, diffusion rates, etc., of implanted substances, doses employed should certainly not massively exceed such levels. It is axiomatic in neurophysiology that whereas a physiologic dose of acetylcholine leads to transient membrane depolarization, a higher dose causes prolonged depolarization. In the case of the biogenic amines, in addition to these considerations, effects on blood vessel tone, and consequent pituitary blood flow, of supraphysiological amounts, also have to be considered.

8. *Parameter of ACTH release studied:*

As a result of lesion and transsection experiments it is well established that different parameters of ACTH release (i.e., circadian periodicity, stress-evoked release, basal levels, and feedback responsiveness) are differentially affected by specific lesions. Therefore, it should also be possible to observe an effect of a given neurotransmitter on one aspect of ACTH release without it affecting any of the others.

9. *Miscellaneous:*

Finally, factors of age, sex, species, end point of pituitary-adrenal function observed, and interval after application of stimulus when pituitary-adrenal function is studied, should also be controlled.

### *Role of Various Neurotransmitters in ACTH Release*

The data presented in Tables I–IV are not meant to be all inclusive. In addition, references are cited only if adequate numbers of animals were studied in a given experimental situation. It should be noted that the available data presented refer only to levels of a given neurotransmitter, not to its turnover. As pointed out above, the latter may well be a more significant measure of available neurotransmitter. Virtually all of the drug studies cited utilizing agents which modify neurotransmitter synthesis or metabolism have measured levels of only one transmitter substance, so that very little data is available on effects of such drugs on transmitter ratios.

1. *Cholinergic transmitters (Table I):*

An increase in plasma corticosteroid levels has been reported in three studies (5, 6, 18) in which similar quantities (5–9.5 μg) of carbamylcholine chloride (carbachol, a long-acting cholinergic agent) was implanted into various central nervous system areas. Such increases were obtained with mamillary body, median eminence and posterior hypothalamic implants, as well as implants in the ventral tegmental area. One of these studies (6) noted suppression of corticosteroid levels after implantation in the septal and preoptic areas and anterior

TABLE I

*Effect of Cholinergic Transmitters on Pituitary Adrenal Function*

| Increased Transmitter Levels (CNS Implantation) | | | | Decreased Transmitter Levels (Atropine Administration) | | | |
|---|---|---|---|---|---|---|---|
| Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness | Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness |
| ↑ (5)<br>C,U, $\boxed{9}$ | N.S. | N.S | N.S, | (20)<br>unchanged<br>S,C,U, $\boxed{16}$ | (20)<br>obliterated<br>S,C,U, $\boxed{18}$ | N.S. | present (20)<br>S,C,U, $\boxed{16}$ |
| | | | | | no effect (20)<br>S,C,U, $\boxed{8}$ | | |
| ↑ or ↓ (6)<br>C,U,0 | | | | | | | abolished (21)<br>I.H.,R,Ap,0 |
| ↑ (18)<br>GP,Ap,0 | | | | | | | |

GP = Guinea pig
R = Rat
D = Dog
C = Cat
B = Baboon

Ap = Pentobarbital anesthesia
U = Unanesthetized
0 = Time of drug administration not specified
$\boxed{n}$ = clock time of drug administration
NS = Not studied
(n) = Bibliographic reference

*Route of Administration*
S = Systemic
I.H. = Intrahypothalamic
I.V. = Intraventricular
I.C. = Intracisternal

hypothalamus, whereas no effect was seen with implants in such areas in another study (5). In the former study, however, changes in adrenal venous corticosteroid levels were used as an end point; in the latter, change from basal corticosteroid levels were used, and these latter were sufficiently low so that a depression of level would be difficult to detect.

The increase in plasma corticosteroid levels following median eminence implantation of carbachol was not blocked by prior dexamethasone treatment, indicating that the terminal "final common pathway" neurosecretory neuron is steroid insensitive (15).

Following systemic atropine administration, decreased levels of cholinergic transmitter were not associated with any changes in basal plasma corticosteroid levels (20) or their responsiveness to stress (20). This experimental situation, however, was associated with obliteration of the circadian rise in plasma corticosteroid levels if atropine was administered at 6:00 P.M., but with normal periodicity if it was administered at 8:00 A.M. (20). Implantation of atropine in the rat anterior, but not posterior hypothalamus, was effective in blocking steroid responsiveness to ether stress (21).

2. *Noradrenergic transmitters:*

(a) *Basal levels.* As is apparent from Table II, studies investigating the role

TABLE II

*Effect of Adrenergic Transmitters on Pituitary Adrenal Function*

| Increased Norepinephrine Levels (Intra CNS or Intraventricular Administration) | | | | Decreased Norepinephrine Levels | | | |
|---|---|---|---|---|---|---|---|
| Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness | Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness |
| ↑ (5)<br>C,U,$\boxed{9}$<br>I.H. | N.S. | N.S. | Present (21)<br>I.H.,R,Ap,0 | ↑ (25)<br>C,U,S,$\boxed{9}$<br>$\boxed{16}$ | (20) (25)<br>No effect<br>C,U,S,$\boxed{8}$<br>$\boxed{18}$ | N.S. | No effect (25)<br>C,U,S,$\boxed{16}$ |
| ↑<br>C,U,0 (6)<br>I.H. | | | Decreased (33)<br>D,I.V.,Ap,0 | ↑ (26)<br>R,U,S,$\boxed{7}$ | (31)<br>No effect<br>R,U,I.C. | | No effect (26)<br>R,U,S,$\boxed{7}$ |
| ↑<br>GP,Ap,0 (18)<br>I.H. | | | | ↑ (27)<br>R,U,S, $\boxed{23}$ | | | |
| ↑ (22)<br>B,U, $\boxed{13}$<br>I.H. | | | | ↑ (28)<br>R,U,S, $\boxed{11}$ | | | |
| ↑ (23)<br>D,Ap,I.V.,0 | | | | No effect (11)<br>R,U,S,q.3h.x8 | | | |
| ↓ (24)<br>R,U,I.V., $\boxed{11}$ | | | | ↑ (29)<br>R,U,I.V. $\boxed{24}$<br>↑ (24)<br>R,U,S $\boxed{9}$ | | | |

of adrenergic transmitters in ACTH regulation have yielded contradictory results. An increase in plasma corticosteroid levels has been observed following implantation of 2–9 $\mu$g norepinephrine* into the hypothalamus (5, 6, 22) (ventro medial or mamillary body areas) amygdala, septum, or hippocampus (5) or ventral tegmental area (18). Intraventricular administration (time of day unspecified) to anesthetized dogs (23) is also accompanied by an increase in plasma corticosteroid levels, whereas A.M. intraventricular administration to unanesthetized rats is reported (24) to be associated with a decrease in plasma corticosteroid levels. Possible reasons for such discrepancies are cited in points 1, 2, 4, and 9 above.

The effects of decreased norepinephrine levels are more difficult to interpret because of considerable variation in experimental design. Most of the studies have utilized systemic administration of a single dose of a-methyl-p-tyrosine, (aMPT), which depletes levels of both brain norepinephrine and dopamine by blocking the action of tyrosine hydroxylase. In two of the studies (24, 26) reporting an increase in corticosteroid levels following either A.M. or P.M. administration of aMPT, corticosteroid levels returned to basal levels well within four hours. This increase in corticosteroid levels was considered to be possibly secondary to systemic effects noted in the animals following drug administration. Another study, however, (27) reported increased plasma corticosteroid levels 9 hours following 11:00 P.M. administration in the rat, but significantly smaller rises 9 hours following 11:00 A.M. (28) drug administration. Administration of aMPT q.3 hr.x8 (11) had no effect on plasma corticosteroid levels. One investigation (29) utilizing 9:00 A.M. administration of FLA-63 (a dopamine-b-oxidase inhibitor, thereby decreasing dopamine conversion to norepinephrine) reports a rise in plasma corticosteroid levels four hours after drug administration. Only one study utilizing intraventricular drug administration has been reported in detail (29). In this instance midnight administration of guanethidine, (which depleted brain norepinephrine levels) was associated with a rise in plasma corticosteroid levels determined 8 hours subsequent to injection. Here, again, points 2, 4, and 9 cited above apply.

It would appear, therefore, that at present there is no conclusive evidence that localized depletion of central nervous system norepinephrine levels is associated with changes in plasma corticosteroid levels. Studies utilizing alpha and beta blocking agents have not been noted in Table II since their mechanism of action in increasing or decreasing adrenergic activity is not via actual changes in level of transmitter substance. There is one preliminary report (30) in a study of human subjects, that administration of methoxamine (an alpha-adrenergic stimulating agent) was followed by increases in plasma ACTH levels. Administration of phentolamine (an alpha adrenergic blocking agent) blocked such responses. Infusion of propranolol (a beta-adrenergic blocking agent) had no effect on ACTH levels. The corticosteroid levels following intraventricular administration of norepinephrine (22) is reported to be blocked by yohimbine, another alpha-adrenergic blocking agent.

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* As either the chloride (6, 18) or bitartrate (5, 22) salt—the latter being employed in view of reports of an excitatory effect of Cl-alone.

(b) *Circadian periodicity.* Systemic administration of aMPT, dibenzyline (an α-adrenergic blocking agent) and intracisternal 6-OH-dopamine (31) (which lowers hypothalamic levels of norepineprine but not those of dopamine) were not associated with any changes in the circadian periodicity of plasma corticosteroid levels.

(c) *Stress responsiveness.* Again, there is inconclusive data in this regard. If the postulated adrenergic inhibition of ACTH release exists (27, 29), then decreased norepinephrine levels should enhance stress responsiveness, and increased levels have the opposite effect. The only experiments reported in connection with decreased norepinephrine levels have utilized either aMPT (25, 26) which depletes levels both of norepinephrine and dopamine, or reserpine (26, 32) which depletes both serotonin and norepinephrine levels. In both of these instances, normal adrenal corticosteroid stress responses were obtained. Increasing norepinephrine levels (intrahypothalamic implantation, amount of transmitter not specified (21)), failed to suppress corticosteroid response to stress in one study in the rat, but was effective in the dog when exceedingly large amounts (5 mg) were administered (33). Administration of iproniazid (a monoamine oxidase inhibitor, which increases both norepinephrine and serotonin levels) did not inhibit the corticosteroid responsiveness of rats to the stress of laparotomy or histamine administration (34).

Phentolamine has been reported to block the normal ACTH rise seen following induction of hypoglycemia, whereas propranolol enhanced ACTH responsiveness to this stimulus (30). Our laboratory (35) has been unable to confirm the reported effects of α- and β-adrenergic blocking agents on hypoglycemia induced ACTH responsiveness. The only apparent difference in these studies was the time of drug administration, 9 A.M. in one (30) and 5 P.M. in the other (35).

3. *Dopaminergic transmitters:*

As seen in Table III, there is little direct data on the effect of increase or

TABLE III

*Effect of Dopaminergic Transmitter on Pituitary-Adrenal Function*

| Increased Dopamine Levels | | | | Decreased Dopamine Levels | | | |
|---|---|---|---|---|---|---|---|
| Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness | Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness |
| ↓ (24)<br>R,U,I.V., $\boxed{11}$ | N.S. | N.S. | ↓ (24)<br>R,U,I.V., $\boxed{11}$<br>↓ (33)<br>D,I.V.,Ap,0 | N.S. | N.S. | N.S. | N.S. |

decrease of dopamine levels and the resultant effect on pituitary-adrenal function. In the experiments assessing stress responsiveness, there was partial inhibition of the corticosteroid response to histamine stress in rats following intraventricular implantation of dopamine (5 μg). The amount of L-DOPA (20 mg) and dopamine (4 mg) necessary to block stress responsiveness in the dog was much greater, and within the pharmacological range. Indirect evidence for a role of dopaminergic transmitters has been adduced from studies in which administration of L-DOPA (which crosses the blood-brain barrier and is converted to dopamine) prevents the rise in adrenal corticosteroid levels following aMPT. (27) However, the same group of investigators reported (29) that use of FLA-60 which decreases norepinephrine levels without effecting dopamine levels, resulted in a rise of plasma corticosteroid levels. In addition they reported that administration of dihydroxyphenylserine (DOPS) to an aMPT blocked animal (DOPS being converted directly to norepinephrine, while dopamine remains low because of aMPT inhibition of tyrosine hydroxylase) also prevented the rise in plasma corticosteroid levels seen following aMPT administration. In all instances, considerations (2) and (3) cited above apply.

4. *Serotoninergic transmitters (Table IV):*

(a) *Basal levels.* Elevation of brain serotonin levels, either by intrahypothalamic injection of serotonin (10 μg) as either the creatinine sulfate (18, 37) or the bimaleinate (25) (to avoid the possible irritant effects of creatinine per se) or by systemic administration of Monase® (which in the cat elevates only brain serotonin levels) is accompanied by elevation of plasma corticosteroid levels. The effective areas in all implantation experiments include the septal and preoptic areas, ventromedial nucleus and anterior hypothalamic areas. Response to mamillary body implantation has been variable. Lowering of brain serotonin levels had no effect of plasma corticosteroid levels.

(b) *Circadian periodicity.* It would appear that either elevation or depletion of brain serotonin levels abolishes circadian periodicity. In the cat, this alteration consists of a decrease in peak corticosteroid levels; in the rat, an increase of trough levels and a decrease of peak levels.

(c) *Feedback responsiveness.* Systemic administration of 100 μg serotonin to adult female (but not male) mice, is reported to block compensatory adrenal hypertrophy, even though this compound does not cross the blood brain barrier.

(d) *Stress responsiveness.* Elevation or depletion of brain serotonin levels had no effect on pituitary-adrenal responsiveness to insulin hypoglycemia (25), piromen (25), cold (39), or shaker (39) stress.

## Conclusions

Despite the paucity of experimental observations and the lack of agreement in some areas, there seems to be ample data to support the thesis that putative central neurotransmitter substances are involved in the regulation of ACTH release. Cholinergic, serotoninergic, adrenergic, and dopaminergic activation are all capable of altering basal ACTH secretion. In the case of the first three transmitters this is associated with an increase in basal steroid levels, in the

## TABLE IV

*Effect of Serotoninergic Transmitters on Pituitary-Adrenal Function*

| Increased Serotonin Levels | | | | Decreased Serotonin Levels | | | |
|---|---|---|---|---|---|---|---|
| Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness | Basal Corticosteroid Levels | Circadian Periodicity | Feedback Responsiveness | Stress Responsiveness |
| ↑ (18)<br>I.H.<br>GP,Ap,0 | Abolished (25)<br>C,U,S $\boxed{8:30}$ | (41)<br>Blocked to low levels<br>M,S,U | No effect (25)<br>C,U,S, $\boxed{16}$ | No effect (25)<br>C,U,S $\boxed{8:30}$ $\boxed{16}$ | Abolished (25)<br>C,U,S, $\boxed{8:30}$ | N.S. | No effect (25)<br>C,U,S, $\boxed{16}$ |
| ↑ (5)<br>I.H.<br>C,U, $\boxed{9}$ | | | | No effect (38)<br>R,S,A, $\boxed{11-15}$ | Abolished (40)<br>R,U,S | | No effect (39)<br>R,U,S, $\boxed{11-15}$ |
| ↑ (25)<br>C,U,S, $\boxed{8:30}$ | | | | No effect (11)<br>R,U,S, $\boxed{9:30-11}$ | | | |
| ↑ (37)<br>I.H.<br>GP,U,0 | | | | No effect (39)<br>R,U,S, $\boxed{11-15}$ | | | |

case of the latter perhaps with a decrease. These effects of a given transmitter appear to be localized to different anatomical areas within the central nervous system. A given transmitter may activate ACTH only in certain areas and not others; a given area may respond to one transmitter and not to another. The only exception would appear to be within the median eminence area, which may serve as a "final common pathway" with regard to synaptic regulation of ACTH release. It is also apparent that a given transmitter may affect one parameter of ACTH release without affecting another. Aside from basal release, alterations in circadian periodicity and stress responsiveness have been the most studied parameters. It would appear that cholinergic and serotoninergic mechanisms are involved in the circadian release of ACTH, whereas stress responsiveness may have cholinergic and adrenergic or dopaminergic components.

These data would fit with the concept of "chemical coding" of ACTH release, similar to that proposed for thirst, hunger, and emotional behavior. (42) That is, the neurons responsible for a given type of ACTH response, (i.e., either stress, feedback, or periodicity) may all be activated by one synaptic transmitter and need not necessarily reside in one discrete area within the central nervous system. A different transmitter may be effective in mediating another type of ACTH response, and again the neurons involved be diffusely represented. The postsynaptic membrane in the median eminence however, as suggested by DeRobertis (43) may have multiple receptor sites for multiple chemical transmitters. This raises the possibility that different naturally occurring stimuli for ACTH release may activate the terminal neurosecretory neuron by chemically independent pathways. It also suggests, as has been noted, that the response of the terminal neuron may be the result of its integration of the effect of two or more different transmitters.

With greater appreciation of the diversity of the areas and transmitters which are involved in neural hormonal regulation, it is to be anticipated that more discrete experimental approaches, in combination with those already available, will substantially advance our understanding in this field.

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# The Assay of ACTH in Plasma

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The significance of scientific advances is dependent upon originality, applicability, and timeliness. A superior rating in each of these categories must be given to the introduction of radioimmune assay of the hormones and particularly ACTH. Others writing in this symposium will point out the importance of radioimmune assay in the determination of a number of other hormones as well as a variety of substances which are present in body fluids in extremely small quantities. To those of us who have worked with ACTH, however, the availability of a technique such as this has been of great importance. This paper will be devoted to a discussion of the measurement of ACTH in plasma and the advances in this effort which have resulted from the introduction of radioimmune assay to the field. When radioimmune assay of ACTH was first introduced the field had essentially reached an impasse. The in vitro and in vivo methods for assay of ACTH, including ascorbic acid depletion and corticosteroid production, had about reached the limits of their capabilites to measure the small amounts of ACTH present in plasma (1–3). Radioimmune assay made possible the measurement of smaller quantities of hormone with greater chemical specificity (4). Later work, as will be described below, has demonstrated the ability of the radioimmune assay technique to measure both biologically active and inactive portions of the ACTH molecule thus leading to greater information concerning metabolism of fragments as well as giving greatly increased sensitivity to the measurement.

### *Standards Used in the Measurement of ACTH*

In attempting to discuss levels of ACTH in plasma, one has to describe the standards used because these have a significant effect upon the final values which may be reported by any method of measurement. An attempt will be made as nearly as possible to relate values given in this paper either to picograms of the 1-39ACTH or milliunits of the 2nd International Standard which contained 1.14 U of ACTH/mg. Berson and Yalow used preparations of Dr. C. H. Li and Drs. A. Lerner and V. Upton which were reported to contain 140 U/mg or 139 U/mg respectively (5). Matsuyama, Mims, Ruhmann-Wennhold and Nelson on the other hand used a Li preparation which was found to have a mean biologic potency of 75.8 U/mg compared with the 2nd U.S.P. reference standard (6). Galskov used a plasma standard which was set at 100 picograms/ml and he emphasized the necessity of using one assay technique with internal comparisons between standards (7). Although theoretically the best standard would be a synthetic ACTH, those preparations which have been generally available have not been absolutely pure and have shown as much, if

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not more, variation than the highly purified preparations obtained from biologic sources. In spite of these problems with standards some agreement concerning plasma ACTH levels has evolved as the techniques have been put into use. As these methods come into wider clinical use, however, it would appear essential that a pure, probably synthetic ACTH standard be made available to laboratories engaged in assay of this hormone.

### *Levels of ACTH in Plasma*

As might be expected from the differences in ACTH reference standards employed by various workers, levels of ACTH reported have varied from laboratory to laboratory. Berson and Yalow describe a diurnal variation with levels at 8:00 to 10:00 A.M. averaging 22 picograms/ml, and those at 10:00 to 11:00 P.M. averaging 9.6 picograms/ml (5). Matsukura et al describe an 8:00 A.M. mean of 70 picograms/ml with a 4:00 P.M. mean of 20 picograms/ml (8). The highest values reported are those in the paper by Jensen and Blichert-Toft in which 8:00 A.M. levels average 101.8 picograms/ml (9). These values were obtained by the method of Galskov in which a reference serum is fixed at 100 picograms. Earlier studies with bioassay had suggested that a diurnal variation in the secretion of ACTH roughly paralleled the diurnal variation in corticosteroid secretion but the relative lack of sensitivity of these techniques allowed only rough demonstration of such variation and generally required extraction of the ACTH prior to carrying out the assay (10). Later studies have clearly demonstrated this variation with approximately 6:00 A.M. peaks (11).

Of further importance in making such determinations is the episodic secretion of ACTH which consists of a series of spikes of secretion rather than a continuous rise and fall in levels occurring over the 24 hr period. Such irregular peaks of secretion were noted in the measurement of corticosteroid secreted in the adrenal veins of dogs in whom chronic cannuli had been placed (12). It was then confirmed for both corticosteroids and ACTH in man (11, 13, 14).

Of considerable interest in the assay of ACTH has been the observation that antibodies may be made to either the C-terminal or N-terminal portions of the molecule and that antibodies to the latter usually show good correlation with bioassay values for ACTH whereas those to the C-terminal end of the molecule tend to show considerable discrepancy, particularly in patients who are producing ACTH at a high rate, as in stress or following the administration of metyrapone. The level of ACTH measured by the radioimmune assay procedure is highly dependent upon the antibody used, just as the absolute values found by the bioassay procedures are dependent upon the purity of the reference standard employed.

The demonstration of a larger molecular weight fraction in plasma which is measurable as ACTH by immunoassay is of importance (15). From the initial data presented, it appears that this is probably a biologically active as well as immunoreactive ACTH which is bound to a larger peptide. One must wonder whether two such corticotropins might have been differentially extracted in those procedures which used extraction of the ACTH from plasma prior to assay. If this is so, then some of the differences which have been noted between levels measured by direct use of plasma as compared with extraction procedures might be at least partially due to differential extraction of these two types of ACTH.

Studies both in rat and man have clearly demonstrated the variations which may occur between measurement of biologically active and radioimmunoassayable ACTH. Besser and coworkers demonstrated in man that bioactive ACTH disappeared from the circulation faster than immunoreactive ACTH following single injection or infusions of the hormone (16). Similarly when ACTH was incubated in blood or plasma the material measurable by bioassay disappeared more rapidly. In their assays two antisera were used, one interacting with the N-terminal and the other with the C-terminal portion of the molecule. In studies carried out in adrenalectomized rats, Matsuyama et al showed fairly good agreement between bioassay and radioimmune assay immediately following adrenalectomy but much higher levels by radioimmune assay seven to 14 days following adrenalectomy (6). As the antiserum used in these studies did not cross react with $\alpha^{1-24}$ACTH it would appear that the immunoassay was measuring C-terminal fragments whereas both methods measured the complete ACTH molecule. Further studies by Matsuyama et al demonstrated that endogenous ACTH in the stressed 14 day adrenalectomized rats had a $t_{1/2}$ of 1.7 min by bioassay and 3.6 min by radioimmune assay (17).

### *Response to Stress and Metyrapone*

Studies carried out with the ascorbic acid depletion assay clearly suggested a rapid response in ACTH secretion following a variety of stresses. Somewhat later bioassay procedures demonstrated significant ACTH secretion with values increasing to 2 milliunits/100 ml plasma one-half hour postoperatively in one series and somewhat less than 1 milliunit/100 ml during surgery in another (10, 18). The lower values were seen in a study in which extraction of the ACTH was performed prior to bioassay although the bioassay procedures were very similar. These studies confirmed previous work carried out in the dog in which an increase in plasma ACTH and cortisol was shown to occur simultaneously (19). Using radioimmunoassay, ACTH has been shown to increase approximately two- to ten-fold following a variety of stresses. Values from the study by Berson and Yalow varied from 40 to 500 picograms/ml immediately postoperatively and showed similar increases to as high as 800 picograms/ml immediately following electroconvulsive shock therapy (5). It is of interest that in these studies, as well as among patients in studies carried out by other workers, individual ACTH samples have not always shown an increase. This apparent lack of elevation of ACTH following stress may result from the fact that ACTH secretion is still episodic through these periods and the peak has been missed in the sampling. As noted by most workers, plasma ACTH concentrations provide more useful information if repetitive sampling is carried out, if they are increased due to some disturbance in primary physiology, or if they are studied following the administration of some substance such as dexamethasone or metyrapone which will influence those levels.

Since the effect of metyrapone to block 11-hydroxylation by the adrenal cortex was first noted it has been employed in a variety of ways to demonstrate the integrity or lack of such of the pituitary adrenal axis. The use of this substance in a test has proven useful in the diagnosis of both pituitary and adrenal disease. The well-known procedure consists of administration of metyrapone in doses of 500 to 750 mg every 4 hrs for 48 hrs with measurement of urinary excretion of 17-keto, 17-ketogenic, or 17-hydroxycorticosteroids. Although this is a useful test it is also a fairly involved one. In those laboratories where plasma ACTH assay is readily available it has also been added to the usual test. Estimation of the 11-desoxycortisol in plasma following a single dose of metyrapone has greatly simplified the test. When this is combined with plasma cortisol measurement and with plasma ACTH measurement it provides a direct and complete measure of the ability of the pituitary to secrete ACTH in response to an appropriate stress. Although the mechanism for the major increase in ACTH appears to be corticosteroid insufficiency resulting from secretion of 11-desoxycortisol rather than cortisol, studies both in the dog and later the rat have indicated that following the administration of metyrapone there is a biphasic increase in ACTH (20, 21). In the rat within two hrs, probably prior to the occurrence of adrenal insufficiency, there is an ACTH increase. A later larger increase, approximately eight hrs after the intravenous injection of the metyrapone, results from the adrenal insufficiency which has been produced. Most normal subjects show a considerable increase in plasma ACTH following either the 750 mg every four-hour schedule or the single administration of 2.0 to 3.0 gm at 11:00 P.M. with determination of the ACTH the following morning.

### *ACTH Levels in Adrenal Insufficiency and Congenital Adrenal Hyperplasia*

The measurement of ACTH in patients with adrenal insufficiency has been one of the easier tasks irrespective of the type of assay used. Due to the high levels of ACTH present (12 milliunits/100 ml plasma or 850 picograms/ml 24 hrs after discontinuance of corticosteroid therapy) it has been possible with a variety of techniques to measure ACTH levels in this condition (22). The availability of radioimmune assay, however, has facilitated the determination of larger numbers of samples and allowed demonstration of the lower levels which may be seen after corticoid administration or immediately following adrenalectomy. The relative difficulty in getting good repetitive estimates of plasma ACTH by the various bioassay procedures resulted in some confusion as to whether removal of the adrenal glands resulted in an immediate increase in plasma ACTH. Studies by Matsuyama et al in the rat demonstrated that the high levels of ACTH (500–1000 picograms/ml) did not occur immediately following adrenalectomy but gradually increased, reaching a high of approximately 1300 picograms/ml after about 2 weeks (6). These levels were maintained for as long as 60–120 days (23). If stressed, however, these adrenalectomized animals were capable of producing much higher levels of ACTH, indicating that the lack of immediate increase was not due to a deficiency in synthetic capability but probably was related to control mechanisms which gradually increase the ACTH level. These data serve as partial evidence for the conclusion, discussed in greater detail elsewhere, that there are two routes for ACTH stimulation (24). One involves the diurnal mechanism and is probably related to the increase in ACTH seen in adrenal insufficiency while the other is the stress mechanism which results in immediate secretion of high levels of ACTH.

A type of adrenal insufficiency in which high levels of ACTH immediately respond to corticoid administration is congenital adrenal hyperplasia with enzymatic deficiency causing decreased cortisol secretion. This is a group of patients in whom a single ACTH determination might quickly suggest the diagnosis and lead to early confirmation and therapy.

### *ACTH Levels in Cushing's Syndrome*

Bioassay of ACTH levels in Cushing's disease consistently suggested increased secretion of ACTH in these subjects. The levels were close to the limits of sensitivity of the methods used however and thus were often not clearly elevated. Some of the lack of elevation may again have been due to the timing of sampling of the ACTH, as shown in studies carried out with frequent cortisol assays, which demonstrated that the episodic secretion of ACTH continues in the patient with Cushing's syndrome (25, 26). As greater experience has been obtained with the estimation of ACTH by radioimmune assay in patients with Cushing's syndrome it has been possible to demonstrate a fairly clear differentiation between the various etiologies of the condition. Patients with hyperplasia almost uniformly show elevated ACTH if multiple samples are taken. Patients with autonomous tumors producing cortisol have zero levels. In some patients who have partial adrenalectomy a recurrence of Cushing's syndrome with much higher levels of ACTH has been demonstrated. Patients who develop pituitary tumors before or after adrenalectomy for their Cushing's syndrome usually have the highest levels of ACTH, sometimes reaching hundreds of milliunits/100 ml of plasma (27). Suppression of plasma ACTH is usually easily brought about in the patient with Cushing's disease either pre- or postadrenalectomy. Some patients who develop pituitary tumors postadrenalectomy, however, can no longer be suppressed by the administration of corticosteroids as the tumor becomes larger and produces more and more ACTH.

Of particular interest are those patients who following the administration of the cortisol analogue dexamethasone have an increase in ACTH secretion rather than a decrease (28). These patients are thought to have an abnormality of control of ACTH such that the dexamethasone blocks the normal action of cortisol and the analogue cannot substitute for cortisol as a suppressant of ACTH secretion. It is of interest that this defect has not been noted in normal individuals who undergo treatment with dexamethasone but only in patients with Cushing's syndrome. Such an apparent association may be evidence that the primary defect in Cushing's syndrome is related to cortisol "receptors" which control ACTH secretion.

### *The Clinical Usefulness of the ACTH Assay*

As radioimmune assay becomes more readily available to the clinician, a number of potential areas of usefulness become apparent. In the patient suspected of adrenal insufficiency the demonstration of a simultaneously elevated ACTH level and low cortisol level might be considered diagnostic. Due to the variation which occurs episodically as well as diurnally in levels of both of these hormones, however, it is probable that a minimum of two samplings would be indicated in most cases to establish the diagnosis. In the patient with adrenal insufficiency secondary to hypopituitarism one expects to find low ACTH and cortisol levels. In these patients the administration of metyrapone (by the single dose approach) with repeat determination of cortisol, 11-desoxycortisol, and ACTH is an accurate and useful approach to the diagnosis. Demonstration of increased ACTH levels and low normal cortisol levels in a patient with elevated androgen secretion is highly suggestive of congenital adrenal hyperplasia. High serum cortisol levels with absent plasma ACTH levels suggest the diagnosis of adrenal tumor producing excess cortisol. It may be expected that increased availability of these tests and particularly the introduction of automated approaches to them will make possible more accurate and more rapid evaluation of patients suspected of having diseases of the adrenal and/or the pituitary gland. Considerable savings in hospital costs should result from the availability of such tests as most of these can be done on an outpatient rather than an inpatient basis.

## Conclusion

A short review of the usefulness of radioimmune assay in the quantitation of ACTH in plasma has been presented. The introduction of this method has made possible the performance of ACTH assays by a number of laboratories and gives promise of making the test generally available to those involved in the diagnosis and treatment of patients with diseases of the adrenal and the pituitary gland. Some of the particular advances in ACTH assay technique and knowledge that have resulted from its availability may be listed as follows: 1. The accurate repetitive determination of normal levels of ACTH has for the first time become possible. 2. Diurnal variation of ACTH which was suggested by a small number of studies by the bioassay technique has been confirmed. 3. The episodic secretion of ACTH has been firmly established. 4. Measurement of fragments of ACTH which do not have biological activity and correlation of levels of these fragments with the biologically active fragments has been possible. 5. Antibodies have been made to various sequences in the ACTH molecule, some of which have biological activity and some of which do not have this activity. 6. It has been possible to follow almost minute to minute plasma ACTH and cortisol levels, thus leading to a better understanding of the episodic diurnal correlation of these hormones. 7. Molecules larger than the normally considered 1-39ACTH which also may have biologic activity have been demonstrated. 8. The development of assays of this type has opened a way to making radioimmune assay of ACTH available to all.

One cannot finish a discussion of this type without paying special tribute to Dr. Berson and his coworkers who, with the introduction of these techniques, as well as the application of them, have contributed greatly to the furtherance of our knowledge of normal physiology and biochemistry and the alterations which occur in diseased states.

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# Glucose Regulation of Insulin Biosynthesis in Isolated Rat Islets*

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Synthesis of insulin in mammalian pancreas has been extensively studied (1–10). A single chain precursor of approximately 80 to 85 amino acids (i.e., proinsulin) is assembled from the amino-terminus of the B-chain to the carboxy-terminus of the A-chain on proinsulin m-RNA. Evidence has been presented that the assembly of amino acids on proinsulin m-RNA occurs almost exclusively on membrane-bound polyribosomes, and that completed proinsulin is released into the cisterna of the endoplasmic reticulum (11). Proinsulin is converted to insulin within membranous particles (12), probably by a combination of trypsin and carboxypeptidase B-like enzymes (13), and each mole of proinsulin gives rise to a mole of insulin and connecting peptide which are stored in secretion granules (14).

Although the synthetic steps are well known, virtually nothing is known about the control of insulin biosynthesis in the pancreas. Since diabetes occurs in patients who develop insulin lack, either relative or absolute, it is conceivable that the lesion may reside in genes which control the rate of proinsulin synthesis. In the anemic disorder $\beta$-thalessemia, for instance, both the $\alpha$ and $\beta$ globin molecules are normal, but there is a deficiency of $\beta$-globin chains. The genetic defect has recently been attributed to a defect in the gene(s) which regulates $\beta$-globin m-RNA production since m-RNA from $\beta$-thalassemic red cells has been measured and found deficient (15).

Before any effort can be made to detect defects in regulation of insulin synthesis, a thorough understanding of the molecular events in hormonogenesis is required. Since alterations of glucose in the physiologic range of 2 to 20 mM have a profound effect on insulin synthesis in vitro (3–6) and may well be the primary determinant of rates of synthesis in vivo, a detailed study of the mechanism whereby glucose controls insulin biosynthesis in isolated rat islets has been undertaken.

## Methods and Materials

### *Islet Preparation and Incubation*

Islets were isolated from adult male Sprague-Dawley rats (300 to 450 gm) as previously described (3), incubated at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4, and gassed continuously with 95% $O_2$–5% $CO_2$.

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### *Polyacrylamide Gel Electrophoresis*

RNA extracted by puromycin and high salt from polysomes was electrophoresed on 3.6 percent acrylamide gels at 22°C as previously described (16). Gels were scanned at 260 nm in a linear Gilford scanner, frozen, and then sliced with a razor in a block with 1–2 mm spaces. The pieces were digested in 300 λ of 1 N NaOH overnight and counted in Instagel® (5 ml).

### *Islet Protein Synthesis Determination*

Total islet protein synthesis was measured by the incorporation of radioactive amino acid into trichloracetic acid precipitable protein. Insulin synthesis was assayed by determining the incorporation of radioactive leucine into insulin antibody precipitates of hormone extracted from isolated islets (3).

### *Materials*

$^3$H-leucine (35.5 Ci/mmole), $^{14}$C-leucine (304 mCi/mmole), and $^3$H-uridine (29.2 Ci/mmole) were obtained from New England Nuclear; sodium deoxycholate, actinomycin D, sodium dodecyl sulfate, sodium fluoride, and 2-deoxyglucose from Sigma Chemical Co.; L-amino acids from Calbiochem; sucrose (ribonuclease-free) from Schwarz/Mann; all other chemicals were reagent grade from Fisher Scientific. NCS tissue solubilizer was obtained from Amersham-Searle and Instagel from Packard.

## Results

### *I. Glucose Control of Insulin Biosynthesis at the Level of Transcription of Islet RNA*

*A. Uptake and incorporation of labeled precursors into islet RNA.* Jarrett, Keen and Track (17) were the first to observe that variations in glucose concentration from 1–2 mM to 10–15 mM in incubation media markedly increased labeled orotic acid incorporation into the RNA of isolated rat islets. They speculated that glucose stimulated insulin biosynthesis may be modulated by transcription of a rate-limiting species of RNA. Sucrose gradient analysis of this newly synthesized RNA (3.6 S) indicated that it was too small for insulin m-RNA, and it was suggested that glucose was regulating t-RNA synthesis (18).

The incorporation of labeled RNA precursors into cellular RNA may be altered by the rate of nucleoside uptake into the cell, its phosphorylation, or by alterations of precursor pool size (19). These parameters were measured on islets incubated with $^3$H-uridine in 2.8 mM and 16.6 mM glucose (20). High glucose increased cellular uptake and phosphorylation of $^3$H-uridine, but an estimate of islet UTP pool size by a double isotope method indicated that glucose did not alter the specific activity of the precursor pool size. Since glucose didn't affect the degradation rate of islet RNA (20), it was concluded that the increased incorporation of $^3$H-uridine represented an accurate measure of increased islet RNA synthesis. Measurement of uridine incorporation indicated that glucose increased RNA synthesis 2- to 3-fold—in all probability a minimal estimate since base composition studies demonstrated that glucose stimulated the overall synthesis of islet RNA species enriched in guanosine and cytosine (20).

*B. Analysis of newly synthesized islet RNA and identification of proinsulin messenger RNA.* Glucose stimulated the synthesis of all species of RNA including heterogeneous nuclear RNA, ribosomal, soluble, and polysomal RNA (20). Since ~90 percent of the rapidly labeled (30 to 60 minutes) polysome-associated RNA is messenger RNA (19), we were interested in determining whether glucose selectively stimulates the synthesis of this RNA species. Islets were incubated in 2.8 or 16.6 mM glucose for one hour in $^3$H-uridine and the polyribosomes isolated as previously described. Analysis of the labeled polysomes on 10–40% linear sucrose gradients is seen in Figure 1. The rapidly labeled polysome RNA was extracted and sedimented, with a peak of 8–10 S on a 5–20% sucrose gradient. Although labeled uridine incorporation was doubled by 16.6

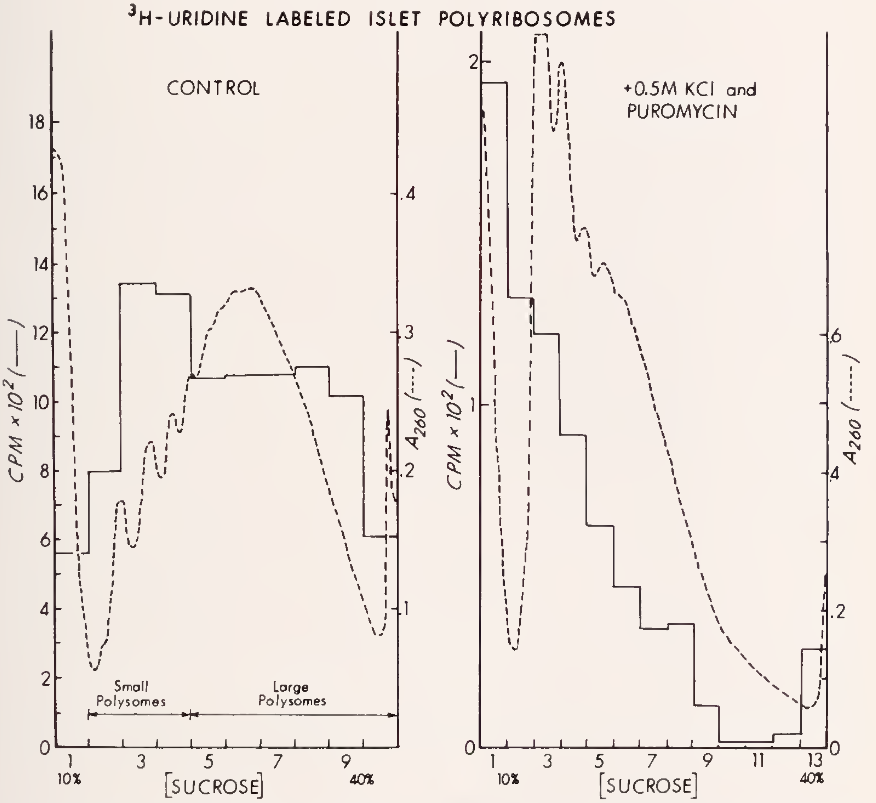

FIG. 1. Rapidly labeled polysome associated islet RNA (m-RNA) on sucrose gradients. Isolated islets (50–100) were incubated (60 min) in $^3$H-uridine, polysomes prepared and analyzed on sucrose gradients as described (11). On the left the $^3$H-uridine incorporated (——) is plotted on the absorbance at 260 nm (– – –) of carrier liver polyribosomes. The right panel is the profile of islet polysomes previously treated with KCl (0.5 M) and puromycin to strip m-RNA.

mM glucose, the sedimentation profiles were not qualitatively different compared to RNA labeled at 2.8 mM glucose. We next sought a more sensitive method for analyzing differences in islet m-RNA. Islet polysomes labeled for 60 minutes with $^3$H-uridine were mixed with isolated liver polysomes (as a carrier) and centrifuged on 10 to 40 percent sucrose gradients and collected in fractions as noted in Figure 1A. Fractions 2 to 4, representing small polysomes, and fractions 5 to 10 representing large polysomes, were pooled, pelleted in an ultracentrifuge, and treated with 0.5 M KCl and puromycin to strip messenger from polysomes (21), as demonstrated in Figure 1B. In preliminary experiments, it was shown that 65 to 85 percent of rapidly labeled islet RNA was released from islet polysomes by this treatment. This RNA preparation was then electrophoresed on porous polyacrylamide gels and scanned at 260 nm for detection of liver messenger RNA (Fig. 2). A significant preparation of the liver m-RNA extracted from large polysomes barely enters the gel whereas most of the liver m-RNA extracted from small polysomes readily enters the gel and migrates toward the anode. Since it has been demonstrated that the larger polysomes contain larger m-RNA (19), these results indicate that the procedures used in this study yield relatively intact m-RNA. The labeled islet m-RNA extracted from small polysomes was heterogeneous; the peak being larger than soluble RNA indicating an average of >100 nucleotides. The m-RNA from large islet polysomes was not as large as anticipated and may indicate some degradation during processing. Using this analytical method, we were unable to detect any difference in m-RNA isolated from islets incubated in either 2.8 or 16.6 mM glucose.

Recently, Palmiter et al (22) have used immunoprecipitation techniques to isolate specific polyribosomes and thereby isolated specific m-RNA. Preliminary studies have been carried out in this laboratory with $^{125}$I-labeled insulin antibody which cross-reacts with proinsulin (3). Thus far, we have not been able to obtain binding of this antibody to rat islet polyribosomes because a large amount of completed soluble insulin molecules adheres to the polysomes, inhibit binding of the specific antibody and cannot be washed off without resulting in polysome breakdown. Using a hamster insulinoma (obtained from Dr. I. Goldfine, NIH) which was assayed at 0.07 U insulin/gm tissue (compared to pancreas at 1 to 2 U/gm), binding of labeled insulin antibody to polyribosomes was readily demonstrated (Fig. 3). This binding was specifically inhibited by adding excess insulin. Maximal antibody binding per unit ribosome (CPM antibody bound/absorbance at 260 nm) appeared in the small polysome region in contrast to the results obtained by Palmiter with ovalbumin synthesizing polysomes. Ovalbumin has a molecular weight of 45,000 compared to 9,000 for proinsulin and consequently, the ovalbumin m-RNA accommodates more ribosomes. It was predicted that proinsulin m-RNA would contain about 300 nucleotides, if monocistronic, and could accommodate at most 3 to 4 ribosomes (11). The data in Figure 3 support the validity of this prediction. At the present time, islet polysome yields are too small to immunoprecipitate sufficient quantities of specific polysomes for m-RNA extraction and analysis.

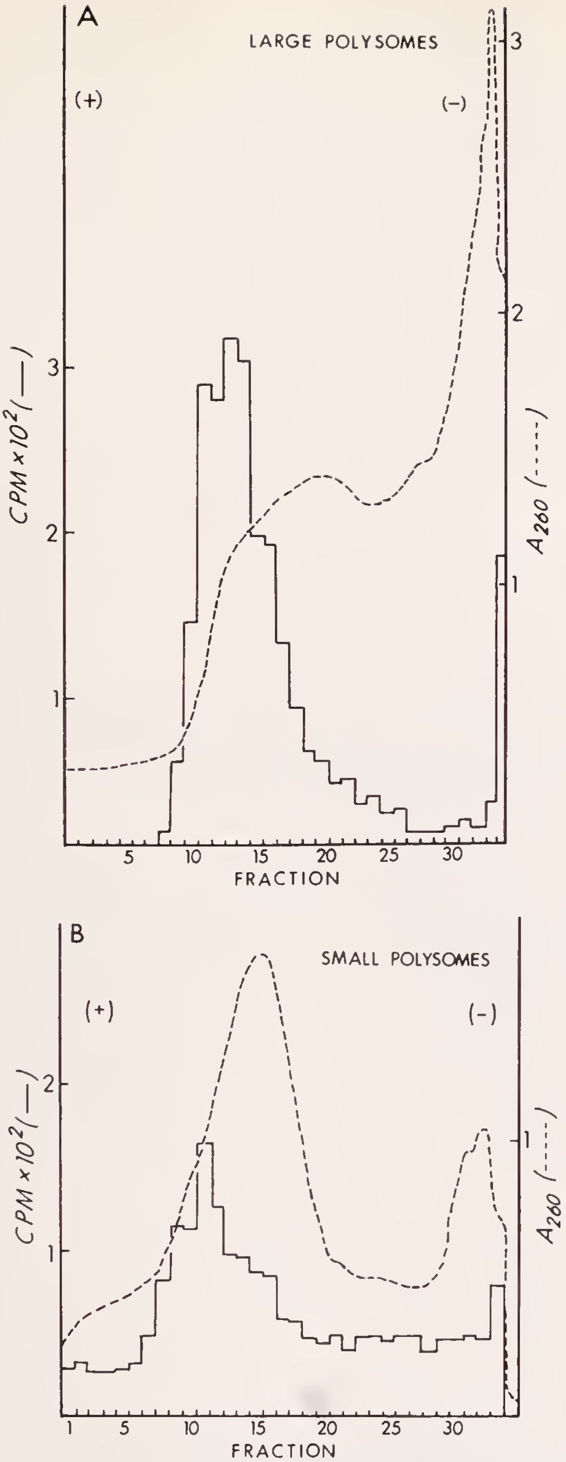

Fig. 2. Islet messenger RNA on polyacrylamide gel electrophoresis. Labeled polysomes from Figure 1 were pooled as indicated, incubated with KCl (0.5 M) and puromycin to strip off m-RNA, then electrophoresed on 3.6% polyacrylamide gels as described in Methods.

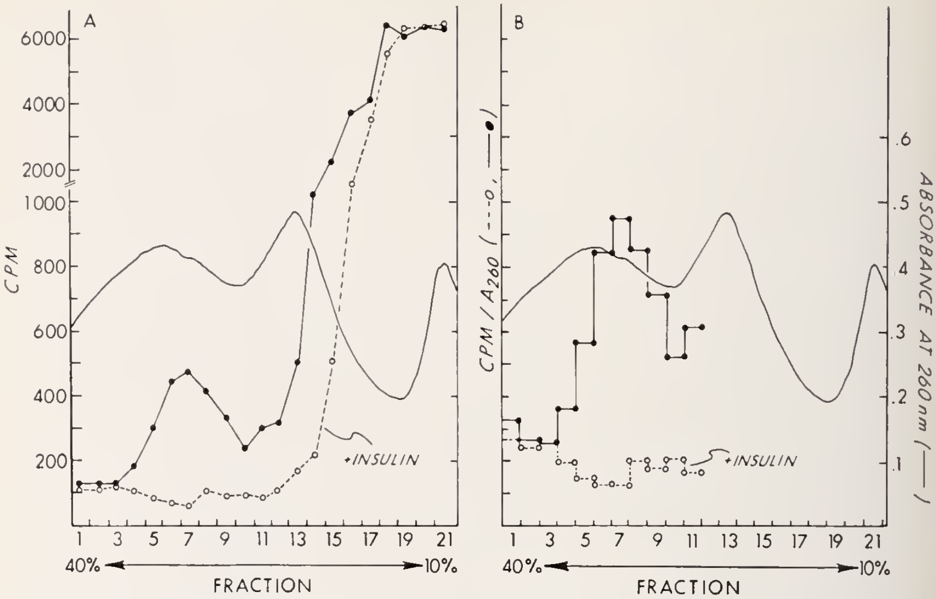

FIG. 3. Binding of $I^{125}$-anti-insulin antibody to polyribosomes from a hamster insulinoma. Anti-insulin $\gamma G$ (45 $\mu g$) was mixed with $I^{125}$ (3 mCi) in 200 $\mu l$ phosphate-saline buffer (pH 7.4) with Chloramine T (4 $\mu g$) for eight minutes. Labeled $\gamma G$ was separated from free iodine on a Sephadex G-25 column. $AI\gamma G\text{-}I^{125}$ was iodinated to a specific activity of 10.4 $\mu Ci/\mu g$. $AI\gamma G\text{-}I^{125}$ was incubated with hamster insulinoma polysomes ± insulin (50 mU) for 60 min at 0°, then analyzed on sucrose gradients as described in Methods.

*C. Evidence that the glucose effect on insulin synthesis is, in part, at the level of transcription.* Indirect evidence that glucose alters insulin biosynthesis at the transcriptional level has been obtained by inhibitor studies (3). It was noted that the effect of high glucose on proinsulin and insulin synthesis by isolated islets was greater during the second hour of incubation than during the first, although the synthetic rate at low glucose was linear through two hours. Actinomycin D added at the beginning of incubation had no effect on proinsulin and insulin synthesis over two hours in low glucose, but blocked the increased rate of synthesis during the second hour in high glucose (Table I). Experiments performed with cycloheximide, a reversible inhibitor of protein synthesis, demonstrated that islets preincubated in high glucose and inhibitor and subsequently washed free of inhibitor and incubated in high glucose alone, synthesized proinsulin and insulin at a more rapid rate than islets incubated de novo in high glucose. If actinomycin D was included in the preincubation period, this stimulation was no longer observed.

These experiments clearly indicated that RNA synthesis is required to observe maximal rates of glucose stimulated insulin biosynthesis. Although glucose may be directing the production of proinsulin m-RNA, we cannot say that this is the rate-limiting event, for r-RNA, t-RNA, or a more labile m-RNA coding for a protein which potentiates translation of proinsulin m-RNA may be

TABLE I

*Glucose-Stimulated Insulin Synthesis in the Presence and Absence of Actinomycin D*

| Glucose | Actinomycin D | TCA Insoluble | AIS Precipitate | AIS/TCA |
|---|---|---|---|---|
| *mM* | *5 μg/ml* | *CPM/μg protein* | | % |
| 2.8 | − | 4451 ± 463 | 162 ± 36 | 3.6 ± 0.5 |
| | + | 3956 ± 301 | 105 ± 30 | 2.5 ± 0.8 |
| 16.6 | − | 7988 ± 884 | 1751 ± 137 | 22.2 ± 1.0 |
| | + | 7479 ± 1172 | 840 ± 75 | 12.3 ± 2.8 |

Isolated islets (10 to 20 per tube in twenty tubes) were incubated for 90 minutes in $^3$H-leucine (100 μCi/ml) and the incorporation into trichloroacetic acid (TCA) insoluble and anti-insulin serum precipitable (AIS) radioactivity determined as described (3). Each result is the mean ± S.E. of five determinations.

involved. Until a specific assay for proinsulin m-RNA in a cell-free system has been developed, this question remains unanswered.

## II. *Effect of Glucose on Insulin Biosynthesis at the Posttranscriptional Level*

Actinomycin D inhibits islet RNA synthesis between 90 and 95 percent (20). Under these conditions, glucose-stimulated insulin synthesis is diminished (see above), but nevertheless, there is a two- to three-fold increase noted in high glucose over a two-hour period (3). Morris and Korner (23) reported similar experiments measuring proinsulin and insulin biosynthesis by Sephadex gel filtration (2). They noted that high glucose increased synthesis about 20-fold over low glucose in control incubations, but only about five-fold in the presence of actinomycin D. These authors attributed the glucose effect solely to a translational event. However, it should be noted that in their study, the effect of glucose increased progressively during the course of incubation similar to what we have reported (3). Steiner and associates have also recently reported that glucose doubled the incorporation of $^3$H-leucine into proinsulin and insulin in the presence or absence of actinomycin D (24). It is clear that, although the magnitude of glucose stimulation of insulin synthesis is diminished when islet RNA synthesis is blocked, glucose can increase proinsulin synthesis on preformed messenger RNA.

We have carried out a series of experiments which suggest that glucose stimulates islet protein synthesis by increasing binding of ribosomes to m-RNA (i.e., initiation of protein synthesis). Islets were incubated for 45 minutes in 2.8 or 16.6 mM glucose, pulsed with $^3$H-leucine for five minutes to label nascent peptides on polysomes, and the islet polysomes isolated with carrier liver polysomes. The isolated polysomes were analyzed on continuous linear sucrose gradients as seen in Figure 4. Glucose markedly increased nascent peptide synthesis (Fig. 4A and B) and produced a shift to increased synthesis on larger polysome aggregates (Fig. 5). If actinomycin D was added during the preincu-

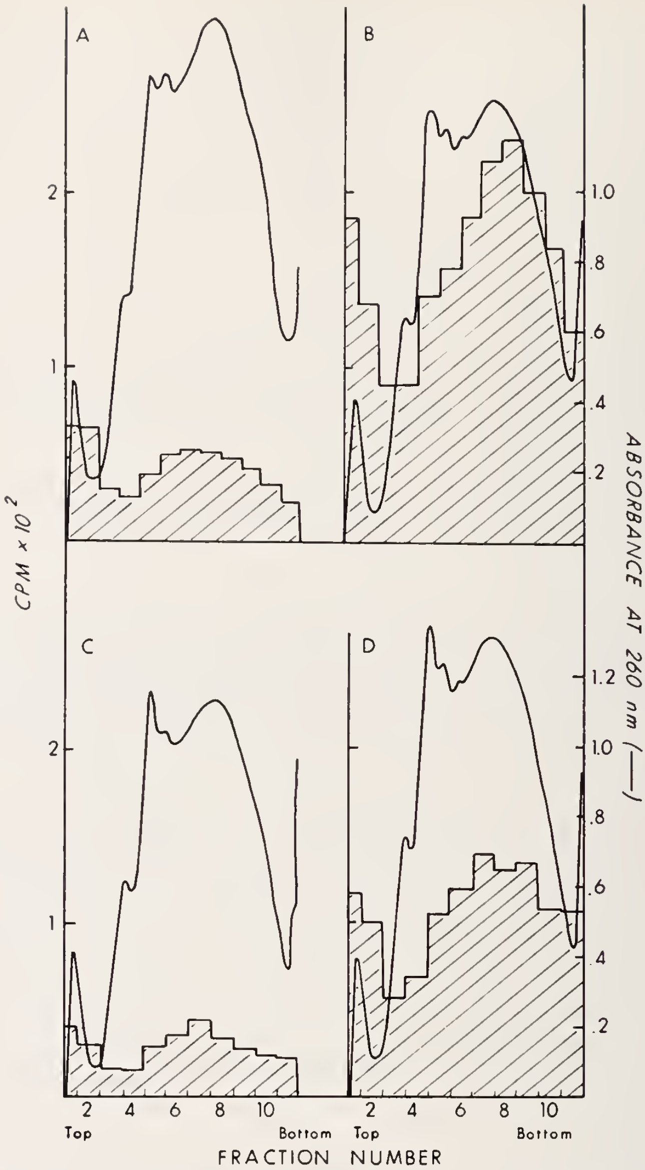

FIG. 4. Glucose activation of islet polyribosomes ± actinomycin D. Isolated islets (50) were previously incubated with 2.8 mM glucose (A) and (C), or 16.6 mM glucose (B) and (D) for 45 minutes, then pulsed with $^3$H-leucine for 5 minutes and polysomes isolated and analyzed as described in Methods. Actinomycin D (5 $\mu$g/ml) was present during the previous incubation in (C) and (D).

bation period no difference was noted in the degree of stimulation by glucose of nascent peptide formation (Fig. 4C and D) or in the shift to synthesis on larger polysome aggregates (Fig. 5). Thus, the number of polysomes synthesizing islet protein, over the time period studied, was increased by high glucose independ-

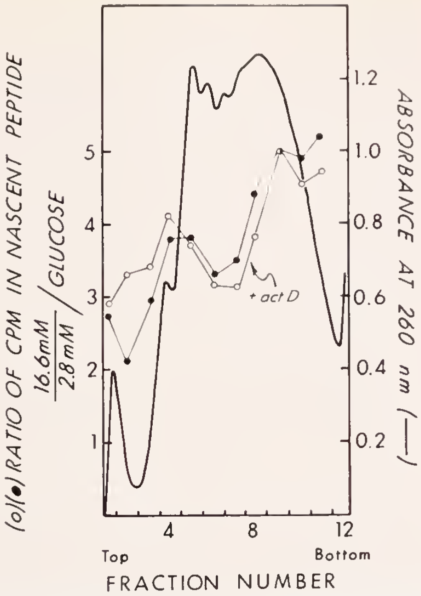

Fig. 5. Effect of glucose (± actinomycin D) on labeling nascent islet peptides. The incorporation of $^3$H-leucine at 16.6 mM glucose/2.8 mM glucose in Figure 3 is plotted on a carrier liver polysome profile.

ent of the formation of new ribosomal, transfer, or messenger RNA. If islets were incubated under conditions known to inhibit the initiation of mammalian protein synthesis (e.g., 10 mM NaF or 22°) then glucose activation of islet polysomes was blocked. Addition of 2-deoxyglucose (16.6 mM) to islets incubated in 16.6 mM glucose, also inhibited the glucose stimulation of polysome activation (25).

Initiation of protein synthesis requires formation of an "initiation complex" consisting of a ribosome, m-RNA, protein initiation factors, ATP and GTP (26). Since ribosomes and m-RNA are present in sufficient quantity, the effects of glucose on islet protein initiation factors, ATP and GTP need to be examined. GTP production has been studied by incubating islets for 50 minutes with $^3$H-guanosine in either 2.8 mM or 16.6 mM glucose. The acid-soluble nucleotide pool was chromatographed on PEI cellulose sheets as previously described (20). As seen in Table II, there was an overall six-fold increased $^3$H-guanosine uptake into the islets incubated in 16.6 mM glucose. In addition, there was a preferential stimulation of GTP formation in high glucose. This data suggests that the islet GTP pool size may be controlled by ambient glucose concentrations. The relationship between glucose concentrations, islet GTP synthesis, and translation of islet proteins remains to be defined.

## Summary

Insulin biosynthesis in isolated rat pancreatic islets has been studied to determine the mechanisms whereby alterations in glucose concentrations alter

TABLE II

*Effect of Glucose on Islet GTP Formation*

| Glucose | $^3$H-Guanosine Incorporated Into | | | | |
|---|---|---|---|---|---|
| | Guanosine | GMP | CGMP | GDP | GTP |
| *mM* | *CPM/µg islet protein* | | | | |
| 2.8 | 0.40 ± 0.03 | 3.86 ± 0.22 | 1.16 ± 0.12 | 1.78 ± 0.13 | 1.90 ± 0.12 |
| % | 4.4 | 42.4 | 12.8 | 19.6 | 20.9 |
| 16.6 | 1.74 ± 0.06 | 7.59 ± 0.86 | 4.56 ± 0.23 | 9.83 ± 1.01 | 28.86 ± 3.28 |
| % | 3.3 | 14.4 | 8.7 | 18.7 | 54.9 |

Isolated islets (10 to 20/tube) were incubated with $^3$H-guanosine (200 µCi/ml) in the glucose concentrations indicated for 50 minutes. An acid-soluble extract was prepared and chromatographed as described in Methods. Each result is the mean ± S.E. of four determinations.

biosynthetic rates. Glucose stimulated the uptake, phosphorylation, and incorporation of islet RNA precursors, as well as the species of RNA synthesized. Islet messenger RNA was stripped from polysomes and analyzed by sucrose gradients and polyacrylamide gel electrophoresis. Islet m-RNA synthesized at 2.8 mM or 16.6 mM glucose was qualitatively indistinguishable by these methods. $I^{125}$-labeled anti-insulin antibody bound to polysomes from a hamster insulinoma. Highest specific activity was in the small polysome region, thus giving the first estimate of the size of proinsulin m-RNA.

Glucose stimulates islet protein synthesis on preformed m-RNA as well. Activation of islet polyribosomes by glucose occurs in the presence of absence of actinomycin D, and occurs by increasing initiation. NaF (10 mM) and lowered temperature (37°C → 22°C) reduce the glucose-stimulated activation of islet polysomes. Islets incubated in 16.6 mM glucose incorporated about six times as much $^3$H-guanosine into GTP as islets incubated in 2.8 mM glucose and the percent of the total nucleotide pool in GTP rose from 21 percent to 55 percent.

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# Pathogenesis of Genetic Diabetes Mellitus: Further Development of a Hypothesis

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In 1967 we presented the hypothesis that genetic diabetes mellitus in all its stages of development is characterized by an absolute or relative insulin deficiency (1). This hypothesis, which contradicted most of the views prevailing since the 1950's, was based on the following observations: 1) The insulin response to glucose infusion, measured under dynamic conditions and in relation to the degree of stimulation, is subnormal in all patients with juvenile or maturity-onset diabetes (2) (Figs. 1–2); 2) A similar deficiency in insulin response is found in subjects with minimal derangement of carbohydrate metabolism (latent or chemical diabetes) (2) (Fig. 2); and 3) The majority of healthy monozygotic twin siblings of diabetic patients (i.e., genetic prediabetics) show the same diabetic type of insulin response to glucose as the above groups (3) (Fig. 3).

We suggested that this diminution of the insulin secretory capacity might be a basic and probably genetic mechanism in the pathogenesis of diabetes (1). A similar deficiency in insulin response was also found in about 20 percent of normal adults (2) (Fig. 4) and children (4) with normal intravenous glucose tolerance. Thus, low insulin response is not restricted to subjects with genetic prediabetes but is also present in a considerable number of people in the normal population. We suggested that these latter subjects represent the prediabetics in the normal population. However, since the majority of these will never develop diabetes, the term "low insulin responder" seems more suitable.

Four major questions emanate from these basic observations:

1. What is the mechanism for the deficient insulin secretion in prediabetes and diabetes?
2. Which mechanisms contribute to the maintenance of a normal glucose tolerance in subjects with deficient insulin secretion?
3. Which are the factors that precipitate clinical diabetes in low insulin responders?
4. Does the deficient insulin secretion bring about any metabolic derangements in low insulin responders?

These problems have been dealt with by our group during the last five years, and some of the results will be discussed in the following.

### *The Mechanism of the Deficient Insulin Secretion*

An important question is whether the secretion of insulin from the pancreatic beta cells is indeed reduced in diabetes, or if the observed hypoinsulinemia reflects an increased retention and destruction of insulin in the liver. This latter

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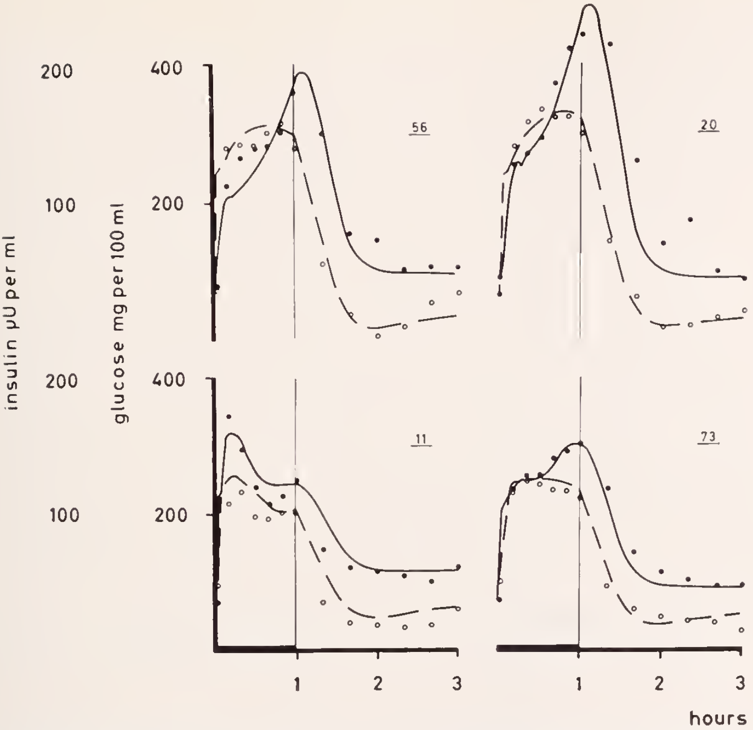

FIG. 1. Results of glucose infusion tests in four healthy subjects with "high" insulin response. Open circles denote blood glucose, solid circles plasma insulin concentrations. The curves have been obtained by analogue computation: whole line, insulin curve; broken line, glucose curve. Glucose infusion between zero and one hour.

alternative is less probable, however, since we and others have shown that insulin response, as measured in portal vein blood, is also subnormal in those instances where it is subnormal in the peripheral circulation (5, 6) (Fig. 5).

It was also suggested that the deficient insulin response might be due to anatomical changes in the capillaries of the islets of Langerhans (thickening of the basal membranes) (7). Such alterations would lead to difficulties in the exchange of glucose and insulin across the capillary wall. Such an explanation seems rather unlikely since, as will be discussed below, acute administration of pharmacologic and physiologic agents may normalize the decreased insulin response. Similar reasons also exclude the possibility that the reduced insulin secretion in diabetes is due to a gross defect in insulin synthesis.

The above discussion made it plausible that the foundation for the disturbed insulin secretion was to be found among those mechanisms which participate in the release of insulin secretion on stimulation with glucose. This makes imperative a discussion of the processes that govern the liberation of insulin in normal subjects.

The generally accepted view until recently was that one or several of the products of glycolysis in the beta cell is the signal that elicits the release of

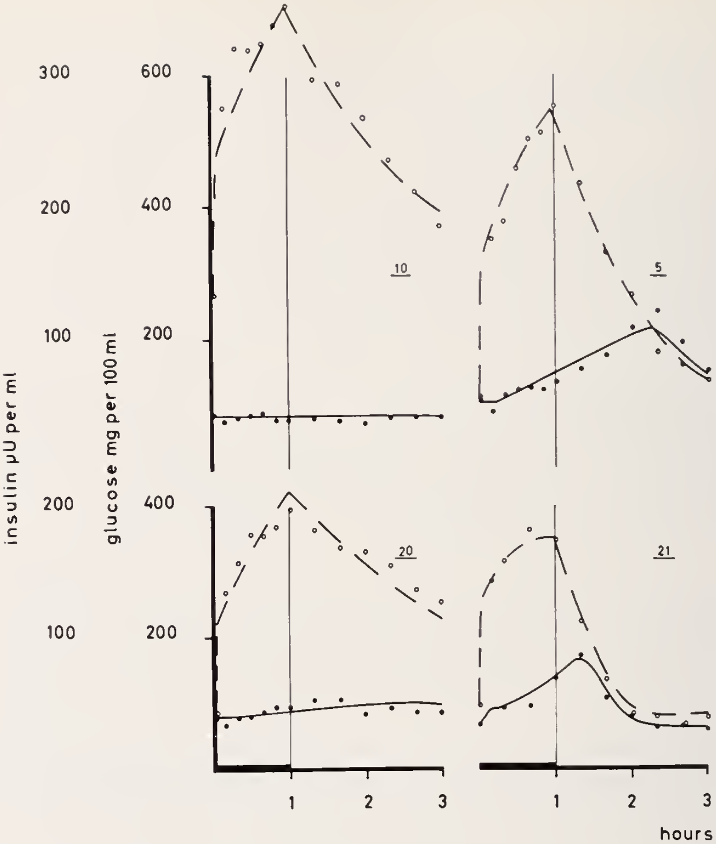

FIG. 2. Results of glucose infusion tests in patients with manifest (top) and decreased glucose tolerance (bottom). For symbols, see Fig. 1.

insulin (8). However, several observations in our laboratory and elsewhere were difficult to explain on the basis of the above view:

1. Kinetic studies show that the insulin response to glucose infusion is a very rapid phenomenon, the maximal secretion rate being reached within 1 to 2 minutes after the start of glucose administration (9) (Fig. 6).
2. The normal insulin response can be inhibited by administering agents which do not influence the glucose metabolism of the beta cells. Thus, the infusion of as little as 1 to 3 ng of epinephrine per Kg body weight reduced by 50 percent the ability of glucose to release insulin (10). Similar inhibition of the insulin response could be obtained with small doses of the beta-adrenergic antagonist, propranolol. This inhibition could be reversed if the subjects were pretreated with theophylline (11) (Figs. 7, 8).
3. More direct evidence against the role of glycolysis in insulin secretion is the finding that glycolytic intermediates accumulate in the beta cells only after long-term stimulation with glucose (12); furthermore, some nonme-

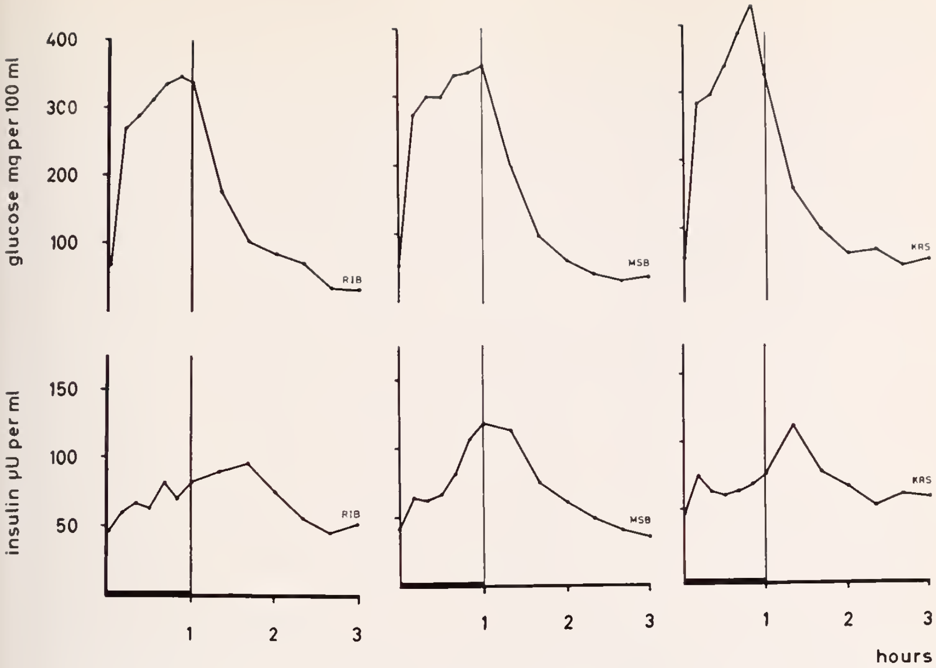

FIG. 3. Plasma insulin response to glucose infusion in healthy monozygotic twin siblings to diabetic patients. Glucose infusion between zero and one hour.

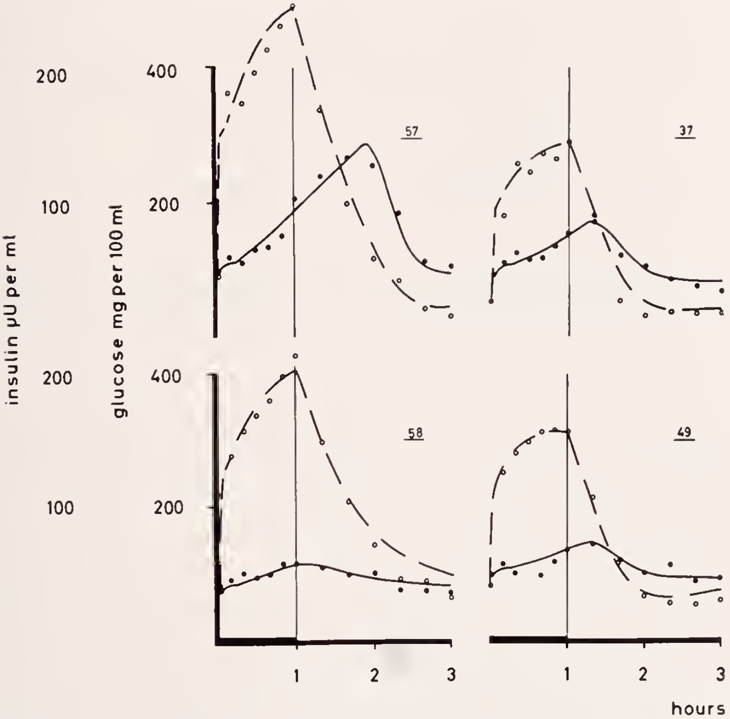

FIG. 4. Results of glucose infusion tests in four healthy subjects with "low" insulin response. Note similarity of insulin response with those in Figs. 2 and 3. For symbols, see Fig. 1.

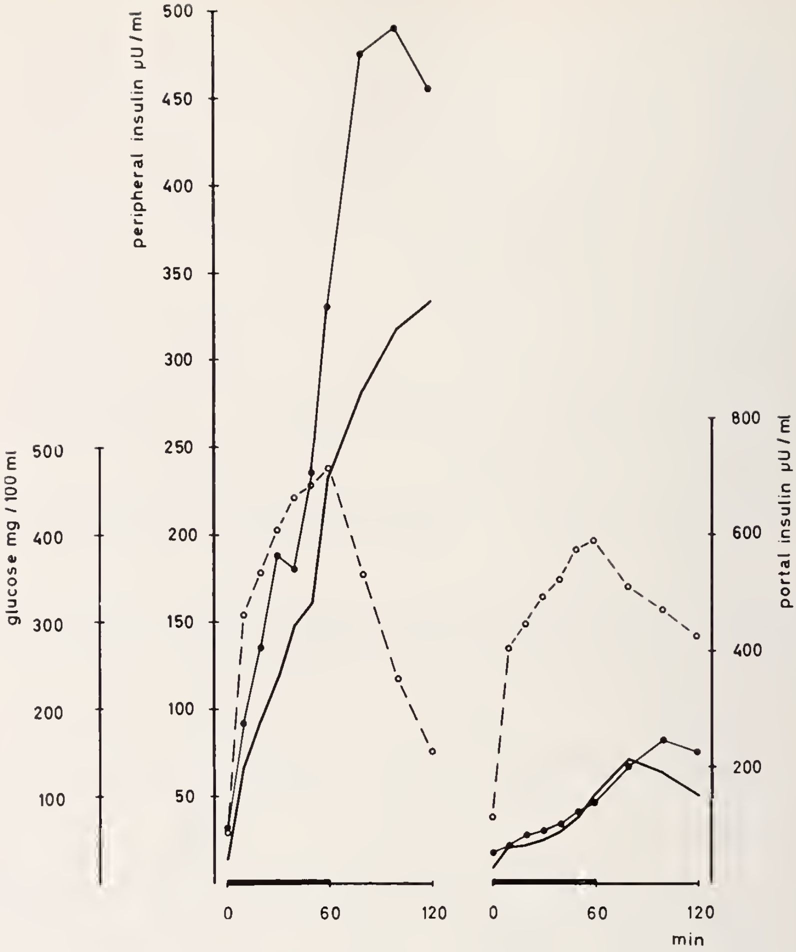

FIG. 5. Insulin response to glucose infusion (0–60 min) as measured in blood from brachial (•——•) and portal vein (——). Blood glucose concentration (•-----•): A. normal subject (left); B. prediabetic subject (right).

tabolizable analogues of glucose and amino acids may induce insulin release (8, 13).

On the basis of our observations we suggested that the secretion of insulin may be controlled by two partially independent processes (14)—a bionergetic system, including the several steps of glycolysis, and a system of cybernetic nature. The latter would comprise a signal system initiated by glucose itself and leading to the release of insulin. According to this hypothesis glucose would, on the one hand, act as a substrate and be metabolized in the beta cell as in all other cells. On the other hand, perhaps by an allosteric action, glucose would act on a cell membrane receptor, inducing an insulinogenic signal (Fig. 9). The mode of transmission of this signal in the beta cell is not clear. It is known that the cyclic AMP system is of importance for the secretion of insulin

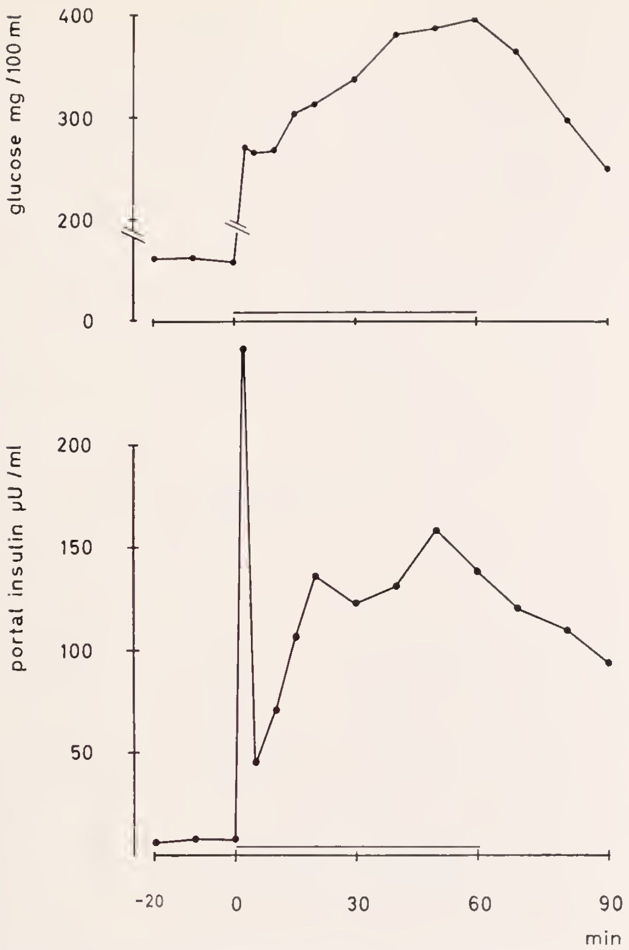

FIG. 6. Changes in portal vein insulin concentration during glucose infusion in a healthy subject. Glucose was injected intravenously and was constantly infused from 0 to 60 min (horizontal line). The first sample was taken at 2 min after the beginning of the glucose injection.

and, consequently, we suggested that the cyclic AMP system could be one of the links of the signal chain. Although some authors (15) have not been able to demonstrate any effect of glucose on islet cyclic AMP, recent studies from our laboratory (Grill and Cerasi, to be published) and elsewhere (15a) show that the cyclic AMP content of the rat islets is increased in the presence of high glucose concentrations.

Irrespective of the nature of the signal chain, our hypothesis implies that glucose has a direct insulin-releasing action while glycolysis in the beta cell plays a secondary role, probably as an amplifier of the glucose signal.

The deficient insulin secretion in prediabetes and diabetes, against the background of the above discussion, may be explained by one of the following

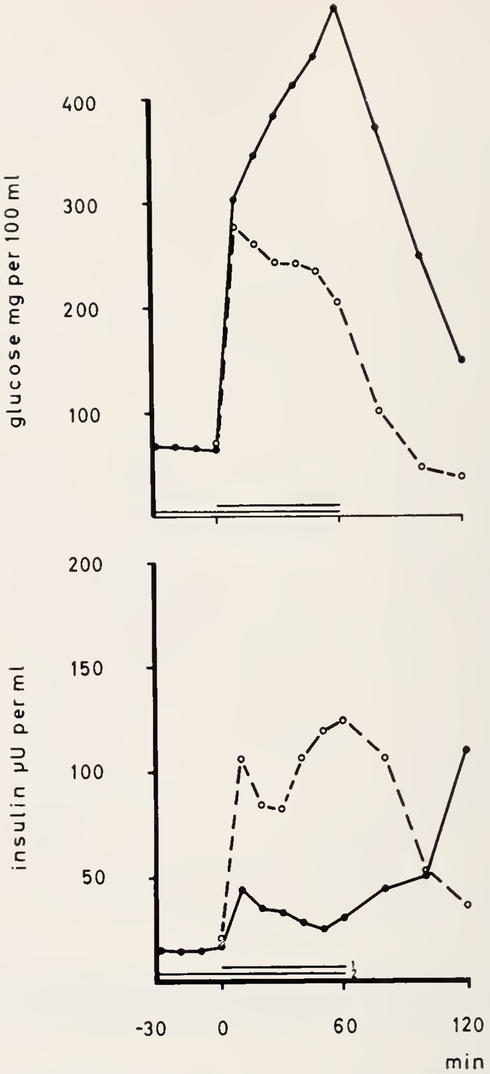

FIG. 7. Effect of propranolol infusion (—30 to 60 min) on the insulin response to glucose infusion (0 to 60 min) in a healthy subject. Broken curves, control experiment; solid curves, infusion of glucose together with propranolol.

alternatives: a reduction of the number of beta cells in the pancreas; a reduction of the amount of insulin in the beta cells; a derangement of glycolysis in the beta cell, hence inability to amplify the glucose signal; or a deficiency in the initiation and/or transmission of the glucose signal.

We and other authors have shown that both diabetics and prediabetics may respond with almost normal insulin secretion when given glucagon (16) or tolbutamide (17, 18) or when glucose infusion was preceded by the administration of theophylline (19) (Fig. 10) or growth hormone (20). These findings exclude the first two alternative explanations given above.

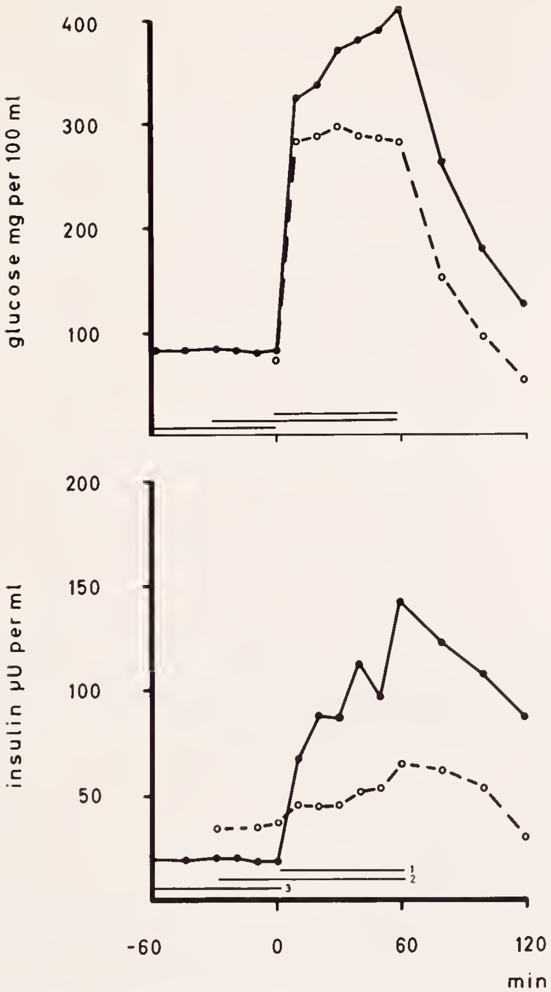

FIG. 8. Reversal of propranolol-induced inhibition of insulin response by aminophylline in a healthy subject. Broken curves, experiment with propranolol (−30 to 60 min) + glucose (0 to 60 min); solid curves, experiment with aminophylline (−60 to 0 min) + propranolol + glucose.

The third alternative, i.e., incapacity to amplify the glucose signal, could also be excluded on the basis of some recent findings. Thus, we have demonstrated that arginine, which increases insulin release by a multiplicative potentiation of the glucose signal (21), exerts the same degree of potentiation in healthy as well as in diabetic and prediabetic individuals. The well-known potentiation of insulin release by intestinal humoral factors was found to be equally effective in these three groups of subjects (22). Finally, preliminary experiments indicate that pretreatment with glucose potentiates the insulin response to a second glucose pulse to the same degree in prediabetics as in healthy individuals. These data favor the idea that the cellular mechanisms which, on activation by amino

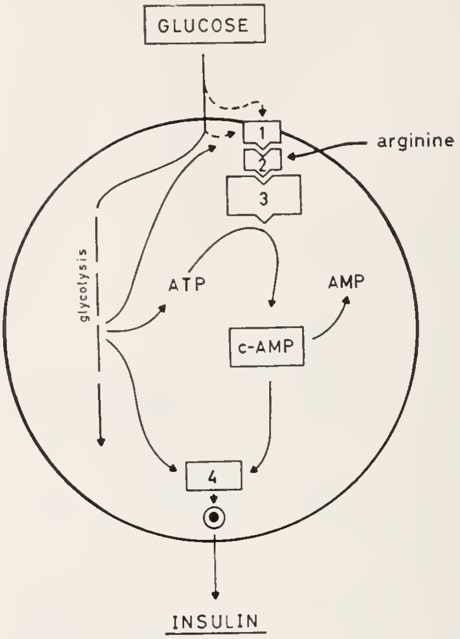

FIG. 9. Suggested mode of action of glucose on insulin release. The signal chain to the right, the metabolic chain to the left: 1. glucose receptor; 2. transmitter unit; 3. adenyl cyclase; 4. phosphodiesterases; 5. insulin-releasing unit.

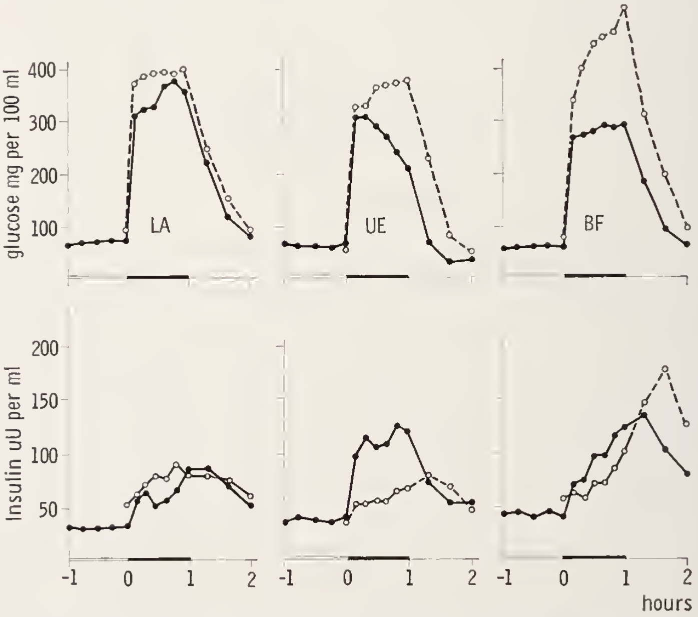

FIG. 10. Effect of aminophylline on glucose-induced insulin release in three prediabetic subjects. Aminophylline was infused from −1 to 0 hours, glucose from 0 to 1 hours. Broken curves: control experiment; Solid curves: experiment with aminophylline administration.

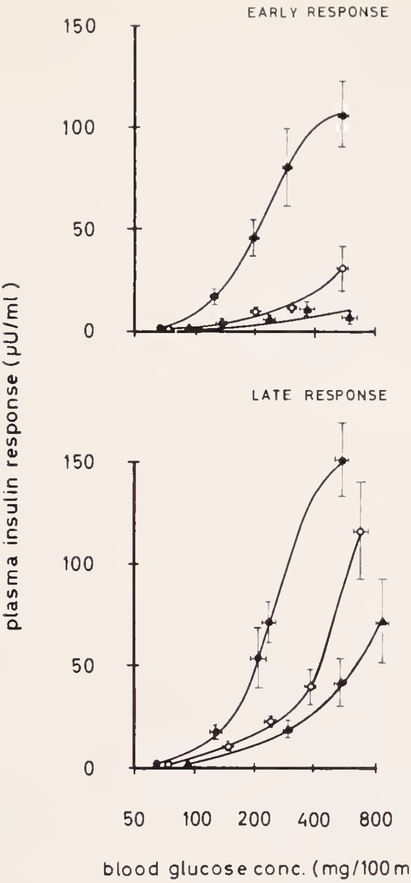

FIG. 11. Glucose-insulin dose-response curves for the early (10 min) and late (60 min) phases of insulin release on glucose administration. Solid circles represent the curves of the control group, open circles those of the prediabetics, and solid triangles the curves obtained in the group of diabetics. Insulin response is given as values above the fasting level, the glycemic stimulus as the logarithm of absolute blood glucose concentrations. Vertical bars denote ± SE of the mean insulin response, horizontal bars ± SE of the mean blood glucose concentration reached at each dose level.

acids, intestinal hormones, or glycolysis in the beta cell enhance the insulinogenic signal of glucose, are intact in diabetes and prediabetes.

These results enable us to delimit the defect in the beta cell in prediabetes and diabetes to the transmission of the insulinogenic signal of glucose, i.e., the fourth of the above alternatives. This assumption is supported by the finding that, in prediabetics and diabetics, a normal insulin response to glucose can be obtained without the above pretreatments, provided that blood glucose will be raised to very high levels. The dose-response relationship between blood glucose and plasma insulin shows a parallel displacement to the right in the prediabetics and diabetics (23). This indicates that the sensitivity—or affinity—of the beta cell receptor for glucose is decreased in diabetes (Fig. 11).

From the above discussion it can be derived that the beta cell defect in diabetes is a very selective one and limited to the transmission of information from the extracellular space. However, by being so strategically located, it impairs grossly the regulation of the secretion of insulin.

### *The Regulation of Glucose Homeostasis in Prediabetes*

Prediabetic subjects exhibit normal glucose tolerance and normoglycemia in spite of markedly reduced insulin levels. From this it follows that prediabetic subjects, in contrast to diabetic patients, have an increased sensitivity to insulin. We have indeed demonstrated that the ability of insulin (both endogenous (24) and exogenous (25)) to diminish the blood glucose level is increased in prediabetes. This raised effectiveness of insulin was not the result of a diminution of the secretion of the insulin antagonists cortisol, epinephrine, and growth hormone.

Previous studies with $^{14}$C-pyruvate suggested that the locus for the increased insulin sensitivity is the liver (26). We have recently analyzed in greater detail the role played by the liver in the regulation of carbohydrate metabolism in prediabetes. By using the technique of liver vein catheterization it could be demonstrated that the basal hepatic glucose output was 25 to 45 percent lower in prediabetic subjects (27) while it was of the same magnitude in diabetics as in normals (28). The hepatic uptake of the gluconeogenic precursors pyruvate, lactate, glycerol, and amino acids was normal in the prediabetics, indicating that the diminished glucose output is brought about by inhibition of glycogenolysis. In contrast, the uptake of these precursors was augmented in the diabetic subjects, a finding in accordance with the well-known stimulation of gluconeogenesis in diabetes.

Administration of small amounts of glucose diminished the hepatic glucose output in normal subjects (27) (Fig. 12). In prediabetics the inhibition of glucose output was far more pronounced despite the minimal insulin response obtained (Fig. 13). No diminution of glucose output was obtained in the diabetics (28) (Fig. 12). These experiments demonstrate clearly that the hepatic sensitivity to insulin is markedly increased in prediabetes. Prediabetic subjects obviously may retain normal glucose tolerance by the aid of such a mechanism. Nothing is known as yet about the cellular mechanisms involved in this hepatic compensation regarding glucose metabolism, nor about the factors that lead to the loss of this compensation and which, hence, precipitate overt diabetes.

### *Conditions That May Precipitate Diabetes in Prediabetics*

It is well established that the occurrence of decreased glucose tolerance and overt diabetes is high in some conditions such as obesity, acromegaly, Cushing's syndrome, etc. These conditions have the common characteristic of demonstrating insulin resistance.

We and others have previously demonstrated that both in obesity (29) and acromegaly (30) subjects with normal glucose tolerance show marked enhancement of insulin secretion. Subjects with the above conditions and decreased

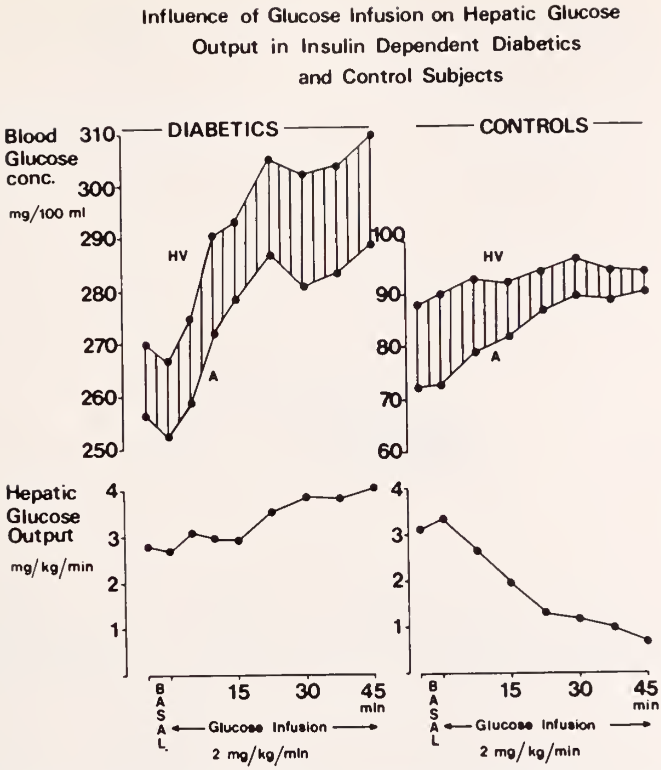

FIG. 12. Influence of glucose infusion on hepatic glucose output in insulin dependent diabetics and control subjects. A: glucose concentration in arterial blood. HV: glucose concentration in hepatic venous blood. Striped area: arteriovenous difference in glucose concentration across the liver.

glucose tolerance had a diminished insulin response as in ordinary diabetics. We drew the conclusion that subjects with normal beta cell function retain normal glucose tolerance in conditions with insulin resistance by markedly increasing insulin output. In contrast, prediabetics are running a greater risk of developing diabetes in these conditions since their capacity to increase insulin secretion is limited.

## *Metabolic Consequences of the Prediabetic State*

The metabolic consequences of extreme deficiency of insulin (overt diabetes mellitus) are well known and involve a broad spectrum of biochemical processes. It may then be questioned whether the insulin deficiency that the prediabetic individual possesses from birth may be accompanied by metabolic manifestations of similar nature as those found so often in diabetes.

Glucose tolerance, by definition, is normal in prediabetes. However, prediabetic individuals as a group show a significantly lower glucose tolerance than subjects with normal beta cell function (2). In addition, in our material of prediabetics followed since 1963, several subjects have demonstrated periodi-

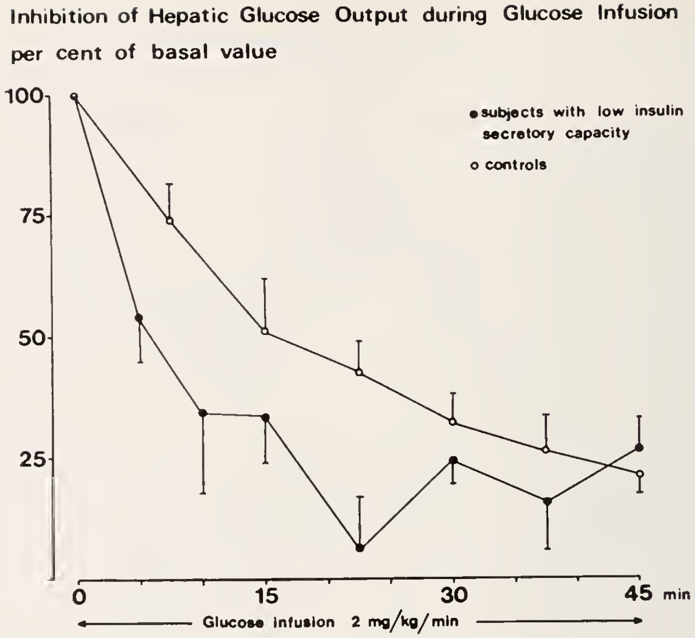

FIG. 13. Inhibition of hepatic glucose output during glucose infusion in low insulin responders (solid circles) and control subjects (open circles). Vertical bars denote SE.

cally decreased glucose tolerance. These observations indicate that glucose homeostasis is controlled with greater difficulty in prediabetic than in normal subjects.

A change in diabetic direction was also observed in the prediabetics regarding lipid metabolism (31). Diabetic patients respond to physical exercise with exaggerated lipolysis (Fig. 14). In a group of prediabetics this lipolytic response measured as increase in plasma glycerol and FFA was greater than in normal subjects but less than in diabetics (Fig. 15). The response in some prediabetics was identical to that found in diabetics. In other words, these prediabetics were normal from the viewpoint of carbohydrate metabolism but diabetic regarding lipid metabolism.

Another observation of interest in this connection concerns the outcome of prediabetic mothers. It is known that newborns of diabetic mothers often demonstrate a higher glucose tolerance than the babies of normal mothers. In addition, malformations and metabolic aberrations occur more frequently in the offspring of diabetics. The presence of such diabetic "stigmata" was found to be higher than normal among the newborns of a limited study group of prediabetic mothers, despite the fact that these women had a normal glucose tolerance throughout the pregnancy.

It is obvious from these isolated observations that insulin deficiency, even in the presence of normal glucose tolerance, may be accompanied by metabolic changes characteristic of overt diabetes mellitus. It has to be added that our

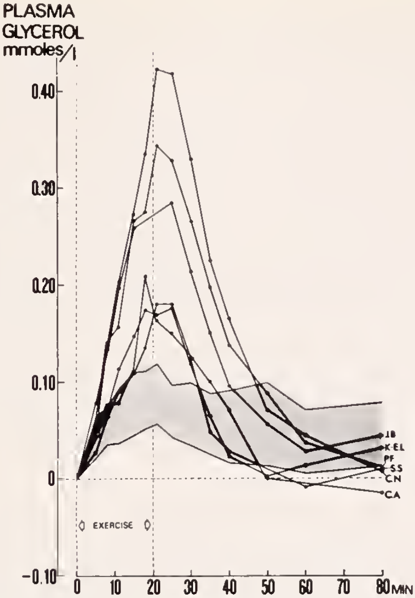

Fig. 14. Changes in the concentration of plasma glycerol during exercise in normal subjects and insulin dependent diabetics. The shaded area denotes the mean ± 3 SD elevation in the normal group. The curves illustrate the individual responses in the diabetic subjects.

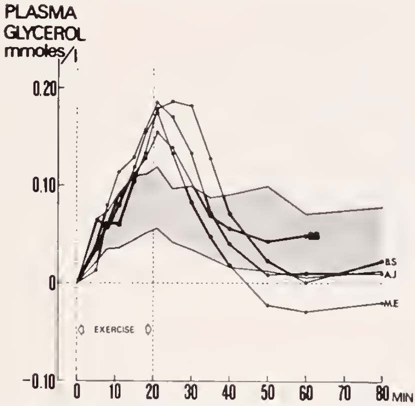

Fig. 15. Changes in the concentration of plasma glycerol during exercise in normal subjects and prediabetics. The shaded area denotes the mean ± 3 SD elevation in the normal group. The curves illustrate the individual responses in the prediabetic subjects.

studies were performed in young prediabetics. We do not know the consequences of lifelong insulin deficiency in older subjects—if we will find in them an increased frequency of the signs of early aging which are so common among diabetics, especially arteriosclerosis and its consequences.

## Conclusions

We have presented a dynamic picture of the pathogenesis of genetic diabetes mellitus, and defined a defect in the beta cell leading to a reduction of insulin secretion—the common denominator of the preclinical and clinical stages of diabetes. It is probable that further characterization of the cellular mechanisms involved may facilitate the development of pharmacologic agents capable of favorably influencing the release of insulin. A future implication of this might be the prevention not only of manifest diabetes but also, and more important, of the metabolic consequences of insulin deficiency.

Our studies have shed some light on the role of the liver in the development of diabetes mellitus. The liver counterbalances the hypoinsulinemia of the prediabetic subject, and the loss of this compensation is probably one of the major factors involved in the precipitation of diabetes. Further elucidation of the mechanisms governing the hepatic compensation may have practical consequences; drugs which favorably influence these mechanisms might be able to prevent the appearance of overt diabetes in prediabetics, but also normalize glucose tolerance in diabetics in whom insulin secretion cannot be corrected adequately.

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# Hyperlipidemia and Diabetes Mellitus: Can Electron Microscopy Help in the Diagnosis?

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It has long been recognized that elevations of plasma triglyceride and carbohydrate abnormalities of varying severity, frequently accompany one another. The relationship between such hyperlipidemia and genetic diabetes mellitus of man, however, remains an unresolved problem. As emphasized in a recent review by Bierman and Porte (1), hyperglycemia frequently occurs in the absence of hyperlipidemia and conversely hyperlipidemia may occur without accompanying hyperglycemia. The overlap of these two conditions is, however, clearly greater than would be expected by chance alone. The well-established fact that hyperlipidemia plays a causative role in atherosclerosis, i.e., macroangiopathy, while on the other hand patients with diabetes mellitus typically develop both microangiopathy and premature macroangiopathy, suggests an added danger from the coexistence of the two states, and adds further impetus to attempts to unravel the relationship between these two conditions.

Studies in this laboratory have recently provided evidence that the microangiopathy of diabetes mellitus may well be independent of the hyperglycemia that characterizes this disease. By means of a technique involving a needle biopsy of the quadriceps muscle coupled with a morphometric, electron microscopic analysis of the width of the capillary basement membrane, it has been possible to quantify the microangiopathic lesion of diabetes mellitus (2–5). With this sensitive procedure, it could be shown that diabetic microangiopathy can be detected in well over 90 percent of adult diabetic subjects. More surprising, this lesion is usually present at the time of onset of hyperglycemia, a finding that suggests that the basement membrane hypertrophy can actually precede the development of hyperglycemia in the diabetic syndrome. In confirmation of this conclusion, we have found that approximately three-quarters of genetically prediabetic subjects demonstrate electron microscopic evidence of microangiopathy prior to the development of overt hyperglycemia or even of a decrease in glucose tolerance (4, 5). Kilo, Vogler, and Williamson (6) have recently confirmed the finding of statistically significant thickening of muscle capillary basement membranes in diabetic subjects. Moreover, despite the fact that their procedure has proven to be less sensitive (6, 7), these investigators have also confirmed the finding that the majority of diabetic subjects have

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thickened basement membranes at the time of onset of hyperglycemia. Recently, our finding of the microangiopathic lesion in the prediabetic subject has been confirmed by Camerini and Bloodworth (8).

The value of employing muscle capillary basement membrane thickening as an independent means of diagnosing diabetes mellitus is greatly enhanced by the finding that the various types of hyperglycemia that are not due to genetic diabetes mellitus, do not produce basement membrane hypertrophy (2), a finding that has recently been confirmed by Danowski (9). As a result, the morphometric method of detecting basement membrane thickening can serve as an independent and relatively specific marker of genetic diabetes mellitus.

Early in the course of these studies it became apparent that use of the capillary basement membrane technique could provide a means of distinguishing a number of distinct syndromes in which hyperglycemia and hyperlipidemia may coexist; and as a result this technique might help to elucidate the relationship between these two clinical conditions. The preliminary results of the studies based on this approach constitute the subject of the present paper. The major conclusion of this study is that hyperlipidemia itself may frequently cause a decrease in glucose tolerance and even severe degrees of fasting hyperglycemia, which are distinct from genetic diabetes mellitus.

## *Hyperlipidemia in Patients with Diabetes Mellitus*

*Diabetic ketoacidosis causing hyperlipidemia.* It is well established that severe hypertriglyceridemia may be precipitated by diabetic ketoacidosis (10); however, it is of interest that the incidence of hypertriglyceridemia accompanying diabetic ketoacidosis is probably less than is generally believed. The Parkland Memorial Hospital experience (11) suggests that only approximately seven percent of patients with diabetic ketoacidosis present with accompanying hyperlipidemia. The underlying cause of this type of hypertriglyceridemia is the best established of any of the related syndromes in that a decrease in insulin has been shown both in animals (12, 13) and in man (14) to lead to a depression in lipoprotein lipase, the enzyme necessary for the metabolism of both endogenous and exogenous triglycerides. As a result, if the diabetic subject has recently consumed a fat-containing meal, chylomicra will constitute the type of triglyceride responsible for the hyperlipidemia. On the other hand, the mobilization of free fatty acids that accompanies the diabetic ketoacidosis may cause both an increased synthesis (15) and release of triglyceride from the liver with a resulting excess of very low density lipoproteins (VLDL) in the circulation.

An example of such a patient is R.S., a 35-year-old black man who, in February 1971, developed the sudden onset of diabetic ketoacidosis associated with severe midepigastric pain, diagnosed as pancreatitis. Serum glucose was 540 mg percent with marked acetonemia. The serum was grossly lipemic with a triglyceride level of 9,140 mg percent, the lipid being carried both in the chylomicra and very low density lipoprotein fractions. Cholesterol was 1,240 mg percent. The patient's ketoacidosis responded promptly to insulin, and a subsequent fasting serum glucose was 263 mg percent, triglycerides 364 mg percent, cholesterol 437 mg percent.

The muscle capillary basement membrane width was 2,616 A ± 236 (diabetic range >1600 A).

This patient, then, demonstrates the typical association of a mixed type of hypertriglyceridemia, consisting both of chylomicra and VLDL, with the acute insulin deprivation of diabetic ketoacidosis. The question of whether this patient might have had acute pancreatitis rather than the genetic diabetes mellitus as the *primary* cause of his insulin deficiency could be readily resolved by the finding of a markedly elevated capillary basement membrane width, a lesion that strongly indicates that he does in fact have genetic diabetes mellitus.

*Poorly controlled diabetes mellitus causing hyperlipidemia.* It has also been well established (16) that diabetes mellitus, even in the absence of acidosis, can, if chronically undercontrolled, lead to hyperlipidemia, due to the accumulation both of exogenous and endogenous lipoproteins. A deficiency of lipoprotein lipase probably is also responsible for this form of hyperlipemia (14).

L.C. is a 44-year-old black woman who has had known diabetes mellitus for three years. She had been treated with 3 grams/day of tolbutamide, but despite the fact that she has never shown any evidence of ketosis, her fasting glucoses chronically ran between 300 and 450 mg percent. On admission to the hospital in November 1972, her cholesterol was 548 mg percent, triglycerides 2,337 mg percent. Both very low density lipoproteins and chylomicra were present.

To determine whether the patient's hyperglycemia was primary, i.e., due to diabetes mellitus, or secondary to the hyperlipidemia, the basement membrane width was measured and found to be 3,146 A ± 139 A, a figure that strongly supports the diagnosis of diabetes mellitus. The patient's therapy was changed from oral hypoglycemic agents to insulin, and she was also placed on a relatively high polyunsaturated fat diet. Her blood chemistries responded promptly and at the time of discharge, fasting glucose was 210 mg percent, triglycerides 240 mg percent, cholesterol 230 mg percent.

While the hyperglycemia and hypertriglyceridemia both were initially treated in this patient, the triglyceride level has remained normal on insulin therapy alone despite the patient having reverted to her previous diet. On the basis of her capillary basement membrane width as well as her response to therapy, the primary clinical problem in this patient is genetic diabetes mellitus, the poor control of which has in turn led to severe hypertriglyceridemia. As noted above, while this association has been well recognized, it should be emphasized that in spite of poor control hyperlipidemia in the diabetic is relatively uncommon (1, 17).

### *Hypertriglyceridemia causing Nondiabetic Carbohydrate Abnormalities*

As will be indicated by the following examples, there is good evidence to indicate that hypertriglyceridemia itself can cause varying degrees of carbohydrate abnormality, which can be distinguished from true genetic diabetes mellitus both by means of capillary basement membrane width as well as by the patient's response to lipid lowering agents.

*Hypertriglyceridemia leading to slight fasting hyperglycemia and decreased glucose tolerance.* L.B. is a 44-year-old black woman who in September 1970 was found to have a fasting serum glucose of 193 mg percent, and a two-hour postprandial value of 264 mg percent. She was diagnosed as having diabetes mellitus, and was briefly treated with tolbutamide. On initial examination her triglyceride concentration was 2,930 mg percent, cholesterol 143 mg percent, no chylomicra were found in the plasma.

The muscle capillary basement membrane width proved to be 919 A ± 125, a figure which in our experience virtually rules out the diagnosis of genetic diabetes mellitus in the adult (3, 5).

The history was obtained from the patient that she consumed 8 to 10 cups of coffee to which a coconut fat cream substitute was added. Hypoglycemic agents were stopped, and the patient was placed on a high polyunsaturated fat diet and advised to avoid using the coconut fat in her coffee. When seen again on 12/4/70, the patient's triglycerides had decreased to 298 mg percent, i.e., to approximately one-tenth the former level. The fasting serum glucose was now normal at 110 mg percent and has remained so.

It has frequently been stated, as might have been suggested by this case, that hypertriglyceridemia, especially of endogenous or mixed types, is accompanied by a high incidence of diabetes mellitus (18–20). It seems far more likely, however, that the hypertriglyceridemia in many such patients represents the primary disease, which perhaps by decreasing tissue sensitivity to secreted insulin, results in a secondary hyperglycemia.

The absence of true genetic diabetes was indicated in this and similar patients (2) by the normal capillary basement membrane width; and this conclusion was supported by the fact that the patient's blood glucose could be reduced to normal, without resorting to hypoglycemic agents, simply by means of a lipid-lowering diet.

*Severe hypertriglyceridemia causing marked fasting hyperglycemia.* Triglyceride levels in 4,000 to 5,000 mg percent range will in our experience frequently cause marked *fasting* hyperglycemia in the absence of diabetes mellitus.

C.W. is a 45-year-old black man who had severe angina thought to be due to atherosclerotic heart disease. A coronary angiogram, however, revealed no evidence of atherosclerosis. When first seen at Parkland Hospital in August, 1972, the patient had severe hyperglycemia, serum glucose 400 mg percent, with a 4+ glycosuria but no ketosis. His triglyceride level was strikingly elevated at 17,000 mg percent, cholesterol, 1,420 mg percent, and both chylomicra and very low density lipoproteins were present in the serum.

The patient's capillary basement membrane width, however, was 1,390 A ± 89, a figure that is below the diabetic level of 1,600 A. (93% of true diabetic subjects will exceed this value (4, 5)). The patient's hyperglycemia was not treated; however, he was placed initially on a completely fat-free diet and subsequently on a high polyunsaturated fat, low carbohydrate diet plus clofibrate, 2 grams/day. When next seen two months later, the patient had gained 21 lbs, i.e., from 205 lbs to 226 lbs. Nonetheless, his triglyceride had decreased to 240 mg percent, cholesterol 230 mg percent, and his fasting serum glucose was 108 mg percent. For the first time in two years, he was free of angina. When next seen in December, 1972, triglyceride was almost within the normal range at 181 mg percent; glucose tolerance test showed a normal fasting value of 123 mg percent with a 1 hr postglucose value of 213 and 2 hr of 228. The 1 hr value is well within normal limits for a man of this age (21), the elevated 2 hr figure being the only residual of his previous severe fasting hyperglycemia.

This patient represents a striking, but rare, example of a patient with marked fasting hyperglycemia, which reverted to normal without any hypoglycemic agents when his hypertriglyceridemia was successfully treated. Again, the initial evidence that the patient's primary disease was hyperlipidemia, not genetic diabetes mellitus, was provided by the finding of a basement membrane width that fell below the diabetic range.

*Hypertriglyceridemia causing pancreatitis and probably permanent hyperglycemia in the absence of genetic diabetes mellitus.* The most severe clinical association between hyperlipidemia and hyperglycemia consists of the destruction of the pancreas that occurs in the occasional patient who has recurrent pancreatitis secondary to severe hypertriglyceridemia.

J.I. is a 32-year-old white man who developed typical signs and symptoms of acute pancreatitis in May 1970. There was no history of alcoholism. Serum amylase was over 700 units, and a fasting serum glucose of 250 mg percent with positive serum acetone accompanied these symptoms. Plasma triglyceride at that time was 2,299 mg percent. No chylomicra were observed. Because of the elevated blood glucose and ketosis the patient was treated acutely with regular insulin.

The capillary basement membrane width, 865 A ± 72, was, however, found to be well within the normal range. Insulin was then discontinued and he was treated only with a high polyunsaturated fat, low cholesterol diet and clofibrate, two grams/day. At the time of discharge, ten days later, the hypertriglyceridemia, while still significant at a level of 495 mg percent, had greatly improved. Fasting glucose was now normal at 124 mg percent, with a 2 hr postprandial value of 173 mg percent. In October, 1971, the patient was again admitted with acute pancreatitis (amylase 6,450 units) and a history of having strayed from his high polyunsaturated fat diet for approximately 6 months. The triglyceride level was once more increased to 1,293 mg percent, and cholesterol was 630 mg percent. Fasting hyperglycemia (393 mg percent) was again present. He was treated with a nasogastric suction, and the hyperglycemia controlled with regular insulin. Oral hypoglycemic agents have subsequently maintained his fasting glucose at a satisfactory but still hyperglycemic level of approximately 150 mg percent, despite a triglyceride concentration that is almost normal at 220 mg percent. Capillary basement membrane width on this admission continued to show a normal value at 1,304 A ± 85.

It has been frequently emphasized that exogenous or fat-induced hyperlipemia may cause acute pancreatitis (22). We have, however, seen a number of patients in whom the hypertriglyceridemia was primarily or exclusively due to an accumulation of very low density lipoproteins, i.e., endogenous triglycerides, but in whom pancreatitis, as in this patient, regularly followed the appearance of severe hypertriglyceridemia.

This case again illustrates the point that, as we previously emphasized (2, 3), hyperglycemia that is secondary to pancreatitis does not cause microangiopathy even as detected by a sensitive morphometric technique. In this patient, we would suggest that the hypertriglyceridemia and accompanying pancreatitis both contributed to the patient's hyperglycemia. Initially, treatment of the hypertriglyceridemia resulted in normalization of the blood glucose; however, with repeated episodes of pancreatitis, insulin reserves decreased and permanent hyperglycemia, even in the absence of hyperlipidemia, resulted. The patient's consistently normal basement membrane width strongly suggests that the primary disorder in this subject is hypertriglyceridemia, not genetic diabetes mellitus.

### *Genetic Diabetes Mellitus Aggravated by Accompanying Hypertriglyceridemia*

Since, as the previous three cases illustrated, hypertriglyceridemia in the absence of diabetes mellitus can produce hyperglycemia, it is reasonable to expect that hyperlipidemia in the patient *with* genetic diabetes mellitus would aggravate the condition leading to poorer control of blood glucose and increasing insulin requirements.

L.S. is a 51-year-old black man who, when first seen in July 1971, had been a known diabetic for two years. Despite treatment with 3 grams of tolbutamide and 100 mg of phenformin per day, fasting serum glucose averaged approximately 290 mg percent. Triglyceride concentration at that time was 953 mg percent, entirely carried in the VLDL fraction.

A capillary basement membrane width of 2101 A ± 184 confirmed the diagnosis of diabetes mellitus.

Because of the elevated triglyceride level, clofibrate, 2 grams/day, and a high polyunsaturated fat diet were added to his regimen. When seen one year later, his triglyceride had decreased to 180 mg percent, and despite no change in his hypoglycemic medication, the fasting serum glucose had decreased to 144 mg percent.

This patient in all probability, then, represents a case of genetic diabetes mellitus whose carbohydrate abnormality was markedly improved after correction of the hypertriglyceridemia.

We have frequently observed, as in this case, that lowering the triglyceride levels in patients with true genetic diabetes mellitus, confirmed by basement membrane hypertrophy, leads to much improved control of their blood glucose levels.

## Discussion

The common denominator in each of the patients presented in this paper is the presence of hypertriglyceridemia and hyperglycemia. As has been frequently emphasized, genetic diabetes mellitus, either in the presence of chronically poor control or during diabetic ketoacidosis can be accompanied by markedly elevated triglyceride levels. Not so well recognized are the syndromes illustrated in this study, in which hypertriglyceridemia may lead to various degrees of glucose intolerance in the absence of genetic diabetes mellitus. The most practical diagnostic tool that we have found in distinguishing between these two general syndromes is the measurement of the width of the muscle capillary basement membrane. By the technique employed in our laboratory (2) a basement membrane width greater than 1,600 A strongly suggests the presence of genetic diabetes mellitus, while a value below 1,325 A makes this diagnosis highly unlikely. As illustrated in this study, hyperglycemia, ranging from decrease in glucose tolerance to permanent fasting hyperglycemia, may be produced by hypertriglyceridemia in the absence of the capillary basement membrane thickening that characterizes genetic diabetes mellitus. Confirmation of this relationship can frequently be obtained by treatment of such patients with lipid-lowering agents with the result that the blood glucose returns to normal in the absence of specific hypoglycemic therapy.

The hyperglycemia that is secondary to hyperlipidemia is relatively easier to treat than is the hyperglycemia of genetic diabetes mellitus, and clearly the disease does not carry the microangiopathic and genetic implications of diabetes mellitus. For these reasons we believe that it is of great clinical importance to make the distinction between these two general causes of coexisting hyperglycemia and hypertriglyceridemia, and to treat the hyperglycemia as a primary disorder in the patient with genetic diabetes mellitus but to direct therapy at the hyperlipidemia in those patients where hyperglycemia is not due to genetic diabetes mellitus.

Finally, when genetic diabetes mellitus and hyperlipidemia coexist, as might be expected, the two abnormalities may aggravate each other, with severe hyperglycemia tending in some patients to elevate triglyceride levels and chronic hypertriglyceridemia aggravating the hyperglycemia. It is apparent that under these circumstances it is important to treat both conditions—the hyperglycemia with suitable oral agents or insulin and the hypertriglyceridemia with a high polyunsaturated fat diet and if necessary with hypotriglyceridemic agents.

The primary purpose of this discussion then is to emphasize the clinical importance of differentiating genetic diabetes mellitus from the primary hyperlipidemic states, which can frequently cause a secondary form of hyperglycemia. As illustrated here the electron microscopic diagnosis of diabetic microangiopathy, by providing an independent marker of diabetes mellitus, has proven to be of great assistance in this differential diagnosis. The distinction between these two disorders with their identical presenting chemical abnormalities can lead both to more accurate diagnosis and to a more rational and effective therapeutic approach to the treatment both of hypertriglyceridemia and of hyperglycemia.

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# Big Gastrin

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Radioimmunoassay has already brought to us a great amount of new knowledge concerning the peptide hormones, much of it unattainable by any other means of investigation presently available. Foremost among the discoveries made with its aid has been that of the heterogeneity of the circulating forms of the hormones; and there is no better example of Berson and Yalow's brilliant use of their own method than the discovery of "big" gastrin, which led directly to its isolation in a pure state.

After the isolation of the hormone gastrin in the form of a pair of heptadecapeptide amides from the antral mucosa of several species and from Zollinger-Ellison tumor tissue (1) it was naturally tempting to suppose that such peptides might constitute the circulating form, but there was nothing to support the idea; if the potency of the circulating hormone were at all comparable to that of the heptadecapeptide, then the amount present in the blood at any moment must be so minute that chemical isolation was not feasible. We turned our attention in 1968 to the possible identification of other forms of the hormone in antral extracts and Zollinger-Ellison tumor tissue.

We and others (2, 3) were aware that in crude extracts of antral mucosa part of the gastrin activity present was in a form larger than the heptadecapeptide; but where the methods used to prepare the extract caused denaturation of the protein (2–5), it was clearly likely that a "big" form of the hormone might result from artefactual binding. Using such a method of initial extraction, a small protein claimed to possess gastrin activity had been isolated from hog antral tissue (6), but others (7) showed that it consisted of the heptadecapeptides loosely bound to denatured protein.

It was reasonable to suppose that a genuine "big" gastrin must incorporate the heptadecapeptide molecule in some manner, either covalently linked to a peptide or protein molecule, or noncovalently bound to a "carrier" molecule—much as oxytocin and vasopressin are bound to the neurophysins (8)—or by simple aggregation. In any of these cases, it was possible that chemically vigorous methods of extraction and purification might disrupt such forms of the hormone, and a method of gentle extraction in the cold was therefore devised.

Small numbers of hog antra were brought from the abbattoir as soon as possible after killing and the mucosa quick-frozen with dry ice. Working thenceforth at 2 to 4°C, batches of this frozen material were extracted with 0.01 M phosphate buffer at pH 6.0. After clarification, the extract was treated with aminoethylcellulose; a small-volume eluate was prepared with 1 M NaCl at pH 6.0 and this applied directly to a large Sephadex G50 column run in 0.01 M phosphate buffer at pH 6.0. The elution profile showed a large protein peak in the void volume which contained much pepsinogen; in the salt zone were the

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heptadecapeptides in the same proportions of sulphated and unsulphated forms as obtained with the boiling water method (5). In a well-defined region about half-way between the void volume and the salt zone there was present a small amount of gastrin activity which gave a delayed, protracted response when injected subcutaneously into conscious pouch dogs. On intravenous infusion the response was slightly slower in onset and more persistent than that seen to heptadecapeptide in the same animals. These results clearly indicated the presence of some kind of a gastrin bigger than the heptadecapeptide; but our attempts to purify it further were unsuccessful.

In 1970, Yalow and Berson (9) reported their discovery that in patients with the Zollinger-Ellison syndrome or pernicious anemia the predominant amount of circulating immunoreactive gastrin was in a form which was considerably larger than the heptadecapeptide.

They subsequently showed (10, 11) that only a small proportion of antral gastrin was in this "big" form (BG) the remainder being "little" (LG), that BG was eluted from adsorption on aminoethylcellulose before heptadecapeptide and so must be less acidic, that BG was unaffected by exposure to boiling-water conditions, and that icewater extraction of antral tissue gave a low total yield of gastrin with no improvement in the proportion of BG obtained. This vital information showed us: (1) the whereabouts of BG relative to void volume and salt zones in a run of crude material on a Sephadex G50 column; this was in fact where we had detected "big" gastrin activity in the "cold" method; (2) that BG was neither "manufactured" nor destroyed by boiling-water extraction, so it was probably a quite robust molecule whatever its precise nature; (3) that BG could be found by reference to heptadecapeptide in chromatographic purification on aminoethylcellulose; and (4) that there was no necessity, but indeed a disadvantage, in using an icewater extraction.

With this invaluable guidance, we reverted to our original method (5), modified it in certain respects and applied it successfully not only to hog antral mucosa, but also to Zollinger-Ellison tumor tissue which we had been accumulating for several years previously. The latter proved far easier to deal with than antral mucosa and there was no difficulty in isolating in a pure state small amounts of a pair of "big" gastrins in addition to the heptadecapeptides (12).

The culmination of Berson and Yalow's brilliant immunologic studies on "big" gastrin was to show that tryptic digestion converted it into the "little" form, suggesting that it might consist of heptadecapeptide joined to a basic peptide by a lysine or arginine peptide bond. The BG peptides we isolated contain no arginine, but two lysine residues; and our colleague Dr. Louis Dancsi in the Department of Organic Chemistry had no difficulty in showing that tryptic digestion of the pure material released two basic peptides and a strongly acidic peptide which had the aminoacid composition of heptadecapeptide and was fully active physiologically. It was, however, ninhydrin-positive, indicating the presence of an N-terminal amino group, whereas heptadecapeptide as isolated in the free form is ninhydrin-negative due to the presence of pyroglutamyl as the N-terminal residue. It is clear that tryptic digestion breaks a peptide bond between lysine and the N-terminal residue of the heptadecapeptide (most probably glutaminyl) in the intact "big" gastrin. This result confirmed and clarified the conclusion of Yalow and Berson as to the structural relationship between BG and LG.

## Discussion

The aminoacid composition of human BG compared with the heptadecapeptide is shown in Table I. The calculated minimal MW is about 3870, compared with about 2200 for the heptadecapeptide. However Yalow and Berson (9) concluded from the behavior of BG compared with that of proinsulin and insulin on Sephadex G50 that its MW was probably about 7000. The BG peptides isolated from Zollinger-Ellison tumor tissue and those from hog antral mucosa run in a position on Sephadex G50 which corresponds to that for plasma BG, while the same is true of their behavior on starch gel electrophoresis; we are grateful to Dr. Rosalyn Yalow and Dr. John Walsh for their assistance with these observations. The discrepancy between the MW of the BG peptides as isolated and their behavior on Sephadex G50 might be explained in one of two ways. Either the peptides behave anomalously on Sephadex—and there are numerous examples of anomalous behavior of substances on Sephadex columns —or they are present in solution as a dimer, which would have a MW about response to intravenous BG substantially exceeds that to equimolar amounts of the BG peptides become available.

Since it is difficult to obtain large numbers of human antra and the amount of BG in them is small (11), it is likely to be some time before isolation of BG peptides from human antral mucosa is accomplished. However, it seems probable that the peptides now isolated are identical with human antral BG, since

TABLE I

*Aminoacid Composition of the Human Gastrins*

| Residues | BG | LG |
|---|---|---|
| ALA | 2 | 1 |
| ASP | 2 | 1 |
| GLU | 8 | 6 |
| GLY | 4 | 2 |
| HIS | 1 | 0 |
| LEU | 3 | 1 |
| LYS | 2 | 0 |
| MET | 1 | 1 |
| PHE | 1 | 1 |
| PRO | 5 | 1 |
| SER | 1 | 0 |
| TRP | 2 | 2 |
| TYR | 1 | 1 |
| VAL | 1 | 0 |
| Total | 34 | 17 |

the pooled material used was derived from about 12 patients; and BG peptide we have since isolated from a single very large tumor has given an amino acid analysis in very satisfactory agreement with that already reported.

Both BG peptides are potent stimulants of gastric acid secretion in conscious dogs. As reported by Grossman (9) who compared ZE plasma and pure heptadecapeptide directly in conscious cats, the rate of onset and offset of a BG response is appreciably slower than with heptadecapeptide. A few experiments we have so far made using Heidenhain dogs receiving continuous subthreshold carbachol to sensitize the pouch indicate that at higher dosage levels the total response to intravenous BG substantially exceeds that to equimolar amounts of heptadecapeptide chiefly because it is more prolonged (Fig. 1). However, the final answer to the question of the relative potencies of BG and LG can best be provided by comparison of secretory responses and plasma gastrin levels, since the more prolonged response to BG suggests that it disappears from the circulation more slowly.

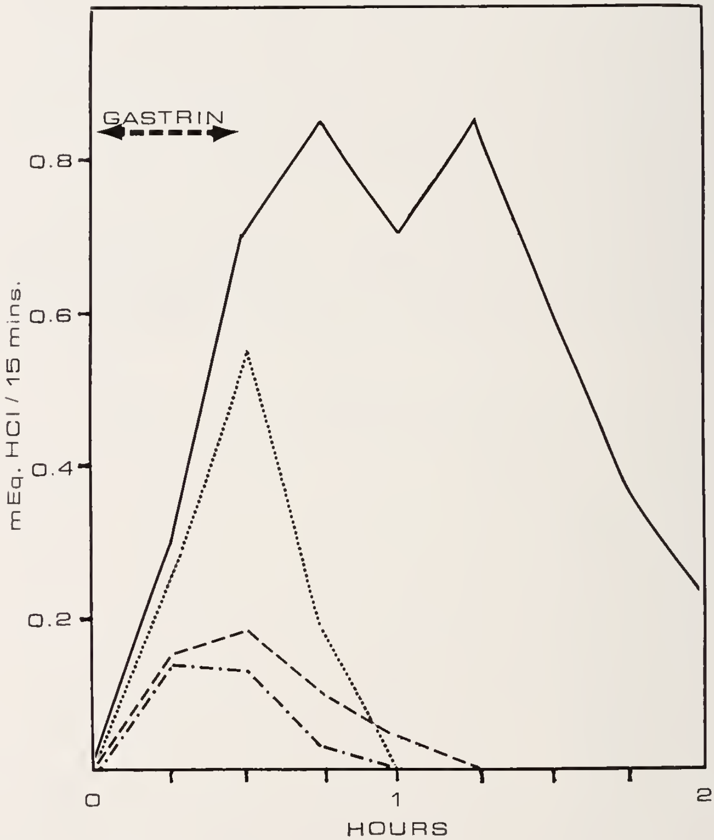

FIG. 1. Response of a Heidenhain pouch to approximately equimolar amounts of BG and LG given by intravenous infusion for the first 30 mins of each experiment. The dog was receiving 1 μg per hour carbachol throughout each experiment. *Symbols:* –·–·–, 0.5 μg LG I; – – –, 1.0 μg BG I; · · · ·, 16 μg LG I; ——, 30 μg BG I.

The physiologic roles and metabolic relationships of big and little gastrin are fascinating problems, in the solution of which radioimmunoassay will play an indispensable part. It is clear that BG predominates, and is sometimes the sole component (9, 10), in the sera of Zollinger-Ellison and pernicious anemia patients. The BG/LG ratio in Zollinger-Ellison tumors seems variable, though the data are fragmentary and much of the material from which they derive was obtained at autopsy. In our pooled material, some of which was obtained at operation, the BG/LG ratio was approximately 1:2. In a very large tumor obtained 18 hours postmortem and now being extracted the ratio appears to be approximately 1:10. A pancreatic tumor obtained at operation and extracted by Berson and Yalow (11) contained about equal amounts of BG and LG; and Dr. Rosalyn Yalow (personal communication) obtained a tumor which contained almost all BG.

In antral mucosa, LG is the predominant form; only a small proportion of BG is present. Yet in the only study known to us of immunoreactive gastrin in post-cibal sera from normal subjects (13) BG was again the major form present. If it proves to be generally true that BG is the predominant circulating form of gastrin in normal subjects, then the reason for the high proportion of LG in antral mucosa is the heart of the problem. Perhaps BG and LG do not in fact stand in any sequential relationship to each other, but are both derived by tryptic-like degradation from a common precursor, and LG is less readily secreted from the gastrin cell than is BG. Another possibility might be that LG is in fact the storage form of the hormone and is largely converted into BG for secretion. Whatever the metabolic relationship of BG and LG, the fragment which distinguishes the two may be identifiable immunologically and possibly chemically (with the aid of radioimmunoassay in fractionation), just as the peptide which distinguishes proinsulin from insulin can be detected in islet tissue and in the circulation; the appropriate BG peptide sequence for raising the specific antibody required will become available when synthesis of BG is undertaken.

Such speculative proposals as these, and many others that might be set down here if space permitted, remind us once again of the major advances not only in endocrinology, but also in medical science in general that have been, and will be, achieved because of Sol Berson's great work. We felt honored to be counted among his friends; like so many others, we mourn his loss deeply.

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# The Development and Application of Antibodies to Gastrin

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The purification and definition of the structure of the gastrin heptadecapeptides (1, 2) produced a great upsurge of interest in the development of antibodies to gastrin. The need for the availability of sensitive and specific antibodies to gastrin was apparent to all involved in the study of the control of gastrointestinal physiologic events, especially to those concerned with the regulation of gastric acid secretion. Precise and specific information was needed concerning the concentration of gastrin in physiologic fluids as well as the localization and distribution of gastrin. Prior bioassay techniques were both woefully insensitive and disappointing in their specificity. Explorations with assay techniques attested to the extremely low concentrations of gastrin in circulating normal serum, and the inadequacy of bioassay in recognizing or estimating physiologic serum gastrin concentrations voiced the need for more sensitive methods of measurement. It was evident that the availability of antibodies to gastrin would provide a means to develop sensitive and specific radioimmunoassay systems for the measurement of gastrin, as had been developed for other polypeptide hormones by Berson and Yalow.

## *Production of Antibodies of Gastrin*

Initial efforts at the production of antibodies to gastrin with immunization of experimental animals with stringently purified gastrin heptadecapeptides were disappointing. It is now evident that the gastrin heptadecapeptides, the form in which most gastrin is present in the antral gastric mucosa, exhibit extremely limited immunogenicity. Subsequently, however, a variety of techniques (3–10) have been developed leading to the successful production of antibodies to gastrin; these methods have permitted the ready measurement of circulating serum and plasma gastrin, as well as the precise localization of gastrin within cells and in tissue extracts.

The methods of production of antibodies to gastrin may be classified in the following manner: 1. Antibodies to the intact, or virtually intact, gastrin heptadecapeptides (as identified by Gregory and Tracy) conjugated to carrier substances; 2. Antibodies produced following immunization using an antigen composed of a portion of the polypeptide amino acid sequence of the heptadecapeptide which had been conjugated to a carrier protein; and 3. Immunization with partially purified preparations of naturally occurring gastrin.

Conjugation techniques have been utilized in order to enhance the immunogenicity of gastrin heptadecapeptides and polypeptide portions of the gastrin

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molecule. In these techniques peptide molecules are either covalently conjugated to carrier proteins utilizing linking agents such as ethyl diethylcarbodiimide, or these peptides are attached, usually noncovalently to particulate materials, e.g., polyacrylamide or other insoluble particles. These kinds of maneuvers have been of great value in increasing the capacity of small, or relatively small, organic molecules to elicit desired immunologic responses. It has long been recognized that, in general, small organic molecules, including polypeptides, are less immunogenic than larger materials, such as foreign proteins. Utilizing conjugate-carrier techniques, various groups of investigators have been successful in producing antibodies with specificity for the intact human gastrin I heptadecapeptide (SHG:1–17) (9), nearly intact human gastrin I (SHG:2–17) (5, 6), the carboxyl terminal tetrapeptide amide portion of gastrin (SHG:14–17) (3, 10), as well as the amino tridecapeptide of human gastrin (SHG:1–13) (9). Each of these methods, using peptides attached to carrier substances, has been successful in the elicitation of antibodies which demonstrate specificity for their individually contained portions of human gastrin heptadecapeptides. It has been amply demonstrated, however, that it is not necessary to utilize conjugation-carrier procedures to elicit antibodies to gastrin. Yalow and Berson were successful in producing high titer highly specific antibodies to gastrin by repeated immunization of guinea pigs with partially purified porcine gastrin (8). These antibodies have been shown to demonstrate a high degree of immunological activity not only with porcine gastrin but with human gastrin, which differs from porcine gastrin only by one amino acid residue.

### *Applications of Antibodies to Gastrin*

Each of the techniques indicated above have been utilized in the application of radioimmunoassay systems for measurement of gastrin and have provided valuable information concerning our understanding of gastrin physiology, localization and distribution in man and experimental animals. Selected examples may be briefly cited. Radioimmunoassay of gastrin has provided a method for the recognition of patients with the Zollinger-Ellison syndrome, (gastrinoma), who characteristically exhibit elevated levels of fasting serum or plasma gastrin (11). Information has been obtained concerning a release of gastrin in response to stimulation, including physiological stimulation such as feeding (12). Although serum gastrin concentrations and the role of gastrin in patients with non-Zollinger-Ellison peptic ulcer disease has been extensively explored by radioimmunoassay of gastrin, many questions still remain. However, from the application of radioimmunoassay methods several important answers are in hand concerning gastrin and peptic ulcer disease. First, fasting serum gastrin concentrations in patients with duodenal ulcer disease appear not to be increased when compared with those of normal individuals (13, 14). On the other hand, in response to physiological stimulation, specifically ingestion of a protein-containing meal, gastrin release in duodenal ulcer patients exceeds that of non-ulcer patients (15–17). There is, in general, probably principally as a result of acid-mediated inhibition of gastrin release, an inverse relationship between rates of gastric acid secretion and fasting serum gastrin concentrations in non-Zollinger-Ellison patients, including those with peptic ulcer disease (14). In some respects, therefore, the relatively normal serum gastrin concentrations in duodenal ulcer patients may reflect a defect at some point in the closed-loop feedback control mechanism regulating gastrin release. Thus the normal serum gastrin concentrations in patients with duodenal ulcer, who usually have increased rates of gastric acid secretion, may represent a relative increase in gastrin level. In addition, duodenal ulcer patients have been shown to be more sensitive to administered doses of pentagastrin and more responsive than non-duodenal ulcer patients in respect to the amounts of acid secreted (18). These observations indicate, in spite of the absence of fasting hypergastrinemia, the potential importance of gastrin in the gastric acid hypersecretory state which frequently accompanies duodenal ulcer disease. The role of the feedback control mechanism in the regulation of gastrin release has also been exemplified by the study of patients with strikingly reduced rates of gastric acid secretion: patients with pernicious anemia, those with gastric atrophy and chronic gastritis without pernicious anemia (particularly those with serum parietal cell antibodies), and patients with carcinoma of the stomach have been shown to have high fasting serum gastrin levels (8, 19–22). Patients with chronic gastritis with circulating antibodies to parietal cells exhibit higher gastrin concentration than those in whom parietal cell antibodies are absent. This appears to reflect the more universal distribution of the gastric atrophic process in patients with chronic gastritis without parietal cell antibodies, as opposed to patients with pernicious anemia and those with chronic gastritis with parietal cell antibodies, in whom the atrophic lesion spares, or only minimally involves, the antral mucosa. High serum concentrations in pernicious anemia may also partially reflect proliferation of gastrin cells in the gastric mucosa, constituting a larger and perhaps more responsive reservoir for release of gastrin (23).

Antibodies to gastrin have been of great value in identifying the distribution and localization of gastrin in various cells and tissues. In their studies of gastrin distribution Yalow and Berson not only identified the presence of gastrin in the small intestinal mucosa, as well as the gastric antrum but shed considerable light on the nature of gastrin in various locations in the gastrointestinal mucosa (24). Although gastrin extractable from the gastric antral mucosa is principally in the heptadecapeptide form, circulating gastrin, as well as relatively large quantities of gastrin in the upper intestinal mucosa, is a larger form of gastrin with a molecular weight of approximately 7000 (big or basic gastrin) (24–26). In more recent studies these investigators identified an even larger species of gastrin (big, big gastrin) which resides principally in the mucosa of the jejunum (27). The results of these studies clearly demonstrate the multiplicity of gastrin species. To date, our knowledge is incomplete concerning the relationship between larger species of gastrin and heptadecapeptide gastrin. From the available data, the most reasonable conclusion regarding the heptadecapeptide is that it may be a structural component of these larger species of gastrin, as evidenced by the fact that trypsin digestion of larger forms of gastrin yields polypeptide fragments indistinguishable from heptadecapeptide gastrin.

The many faces of gastrin are reflected not only in the variety of molecular species of the hormone but also in results of studies of the types of cells shown to contain gastrin. Utilizing immunofluorescent techniques, gastrin has been demonstrated to be contained in granules which pack the cytoplasm of endocrine cells scattered along the course of the antral pyloric glands (28). These cells have been designated as "gastrin cells" and by some authors as "G cells". Utilizing antibodies to gastrin it has also been possible to identify gastrin in the pancreas: cells containing gastrin in their cytoplasmic granules have been identified in the periphery of pancreatic islets, and these cells exhibit the staining characteristics of delta cells (29–30). Pancreatic islet gastrin cells stain positively with the Hellman-Hellerstrom stain (31) (an argyrophilic silver stain), whereas, in our studies antral gastrin cells are Hellman-Hellerstrom negative. Little is known about the structural form in which gastrin exists in the gastrin (delta) cells of the pancreatic islets. The gastrin cell types in the antral mucosa and pancreatic islets appear to differ, at least in respect to their affinity for silver salts in the Hellman-Hellerstrom argyrophilic staining technique. These results may suggest the possibility that the form in which gastrin exists in the pancreatic delta cells differs from that in the antral gastrin cell, in which heptadecapeptide gastrin is the dominant molecular species. Thus, it is conceivable that recognizable differences in cell types in the antrum and pancreas may be paralleled by structural differences in the gastrin molecule similar to, or perhaps differing from, those already described by Yalow and Berson.

### *Separation Techniques for Radioimmunoassay*

In the radioimmunoassay measurement of any substance, including gastrin, it is necessary to develop and utilize a satisfactory method for the separation of antibody-bound from antibody-free radiolabeled tracer material. Many techniques have been applied in the radioimmunoassay of gastrin. These have included the double antibody method, the charcoal absorption method, salt precipitation techniques and column chromatographic separations. In 1970, Drs. Yalow and Berson successfully introduced the use of the Rohm and Hass Amberlite resin CG-4B for the separation of antibody-bound from antibody-free $I^{125}$ gastrin in their studies utilizing antibodies to porcine gastrin and $^{125}I$-porcine gastrin as the tracer material (8). This technique takes advantage of the strong net negative charge of the antibody-free gastrin molecule which becomes firmly attached to the anion-binding resin CG-4B, thus readily separating it from antibody-bound gastrin which is not bound by the resin to any significant degree. The use of CG-4B for separation is equally applicable to radioimmunoassay systems utilizing antibodies to human gastrin I, $^{125}I$-human gastrin I as a radiolabeled marker, and human gastrin I for calibration diagrams. We have found that CG-4B possesses many advantages over the use of double antibody systems, particularly in respect to its simplicity and facility of use. The anion-binding resin CG-4B may be utilized with antibodies to human gastrin I in the following manner. Incubations are performed in 10×75 mm disposable glass tubes adjusted to a final volume of 2.5 ml by addition of 0.02 M barbital buffer, pH 8.4 containing ovalbumin 2.5 mg/ml (EA-Barb). $^{125}$I-human gastrin I (residues 1–17) ($^{125}$I-SHG), approximately 6000 counts per minute, in 0.2 ml EA-Barb is included. The incubation mixture contains 0.2 ml of antiserum to human gastrin I which is diluted sufficiently in EA-Barb so that a bound over free ratio of $^{125}$I-SHG is achieved which is between 0.6 and 1.0 (final dilution usually 1:500,000 to 1:1,500,000). Known quantities of gastrin for the construction of calibration curves as well as unknown samples, e.g., serum or tissue extracts, in appropriate dilutions, in 0.2 ml are included. Immune incubations are conducted at 4°C for 2 to 5 days. Following completion of incubation the anion-binding resin CG-4B may be readily used to separate antibody-free from antibody-bound $^{125}$I-SHG. CG-4B, at a concentration of 100 mg/ml, is suspended in 0.02 M barbital buffer, pH 8.4. Two tenths ml of the CG-4B-containing suspension is added to each incubation tube, after which the tube is gently mixed for 5–10 seconds using a Vortex mixer. The tubes are then centrifuged for 20 minutes at 1000 × G at 4°C: antibody-bound $^{125}$I-SHG remains in the supernatant and antibody-free $^{125}$I-SHG which is bound to the CG-4B particles, is readily precipitated. Calibration diagrams may be established using bound over free ratios, with subsequent application of these calibration diagrams for the measurement of gastrin contained in samples of unknown gastrin concentration (Fig. 1). Introduction of CG-4B has been a most useful adjunct to the development of radioimmunoassay for gastrin. Several characteristics of the behavior and use of CG-4B ought be emphasized. The pH 8.4, as selected by Yalow and Berson, represents an ideal pH for the activity of CG-4B in binding gastrin. Many radioimmunoassay systems are conducted at

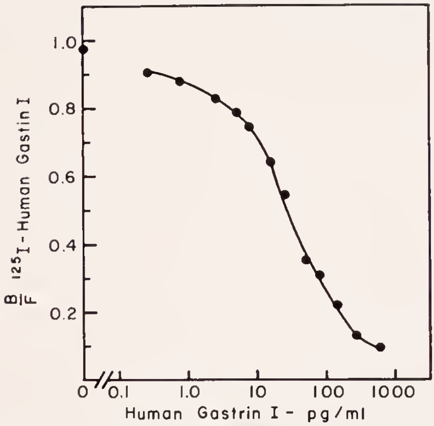

FIG. 1. Calibration curve demonstrating ratios of antibody-bound $^{125}$I-human gastrin I to antibody-free $^{125}$I-human gastrin I in the presence of varying amounts of non-radiolabeled human gastrin I. Antibodies to human gastrin I (SHG:2–17) were utilized at a final dilution of 1:500,000. Values expressed on the horizontal axis refer to pg gastrin per ml incubation mixture; sensitivity level approximately 0.3 pg/ml.

lower pH, e.g., pH 7 or 7.4: at lower pH such as these the affinity of CG-4B for gastrin is greatly reduced, and its capacity to serve as an effective means for separating antibody-free from antibody-bound $^{125}$I-SHG is enormously diminished. The maximum ratio of serum to total incubation volume, following the addition of CG-4B suspension, is 1:12. Attempts to utilize larger amounts of serum will result in loss of linearity in the estimation of the concentration of serially diluted serum samples and spuriously low estimations of the gastrin content of serum samples. Finally, inclusion of large amounts of heparin (like gastrin, a strongly negatively charged molecule) inhibits CG-4B binding of gastrin, and again, may thereby result in lower than true estimations of gastrin content: this has led us to prefer the measurement of gastrin in serum rather than heparinized plasma when using the CG-4B separation technique.

### *On the Specificity of Antibodies to Gastrin*

Cholecystokinin-pancreozymin (CCK-PZ) has been shown to possess the same carboxyl-terminal pentapeptide amino acid sequence as the gastrin heptadecapeptides (32). This limited region of structural identity would be anticipated to provide potential opportunities for immunological cross-reactivity with CCK-PZ by various antibodies to gastrin. In fact, limited immunological cross-reactivity of gastrin antibodies with CCK-PZ has been identified by a variety of investigators. Fortunately, with antibodies produced by immunization with partially purified porcine gastrin or with the complete, or virtually complete, heptadecapeptide gastrin-carrier conjugate, the degree of immunological cross-reactivity is minimal and does not interfere with satisfactorily specific radioimmunoassay of gastrin. On the other hand, antibodies produced to the carboxyl-terminal tetrapeptide amide portion of gastrin exhibit equivalent immunological reactivity with gastrin and cholecystokinin-pancreozymin (3, 10). This characteristic makes these antibodies incapable of distinguishing between cholecystokinin-pancreozymin and gastrin and severely limits their applicability for satisfactory radioimmunoassay of gastrin. It has been possible to elicit gastrin antibodies with exclusive specificity for gastrin, with no detectable immunologic cross-reactivity with cholecystokinin-pancreozymin, by immunization of rabbits with the amino tridecapeptide of gastrin (SHG:1–13) covalently conjugated to bovine serum albumin (9). These antibodies do not recognize or react with CCK-PZ (Fig. 2) nor with the carboxyl-terminal tetrapeptide amide portion of gastrin. It should be emphasized, however, that these exclusively gastrin-specific antibodies provide no useful advantage over those types of antibodies used in the radioimmunoassay of serum or plasma gastrin which have minimal cross-reactivity with CCK-PZ.

Although not necessary for routine radioimmunoassay for gastrin, antibodies with absolute specificity for the unique portion of the gastrin molecule (SHG:1–13), may have advantages in asking certain kinds of experimental questions. These antibodies may be of value in sorting out the structural complexity of circulating gastrin in its various forms, both the larger forms of gastrin and possible circulating degradative products which may be both

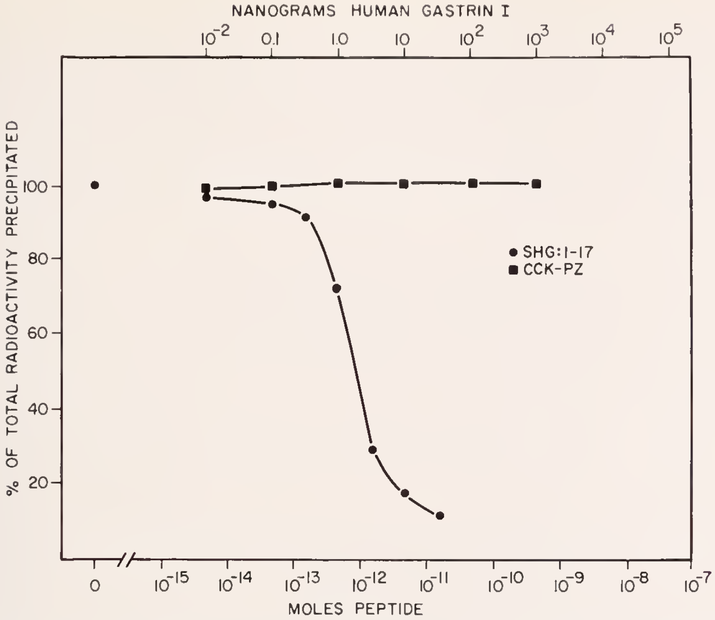

FIG. 2. Calibration diagram indicating the percent of total [$^{125}$I] SHG:1–17 precipitated by addition of goat antibodies to rabbit γG-immunoglobulin in the presence of rabbit antibodies to the human I gastrin amino tridecapeptide (SHG:1–13). Results indicate antibody binding of heptadecapeptide gastrin but no detectable binding of CCK-PZ.

smaller than heptadecapeptide gastrin and perhaps of reduced biological activity. Secondly, antibodies to the amino tridecapeptide portion of gastrin have been of use in studies involved in the localization of gastrin by immunofluorescence. Immunofluorescence, unlike radioimmunoassay, is principally a qualitative rather than quantitative technique. Its demands for immunologic specificity are perhaps even greater than those required in radioimmunoassay. An example which may be cited is that of the identification of gastrin in granules contained in the cytoplasm of delta cells of normal human pancreatic islets. In these studies, gastrin was identified in the cytoplasm of delta cells using direct immunofluorescence techniques. Specific immunofluorescent staining was identified with use of fluorescein-labeled antibodies to human gastrin I (SHG:2–17) and identical results were obtained using fluorescein-labeled antibodies to the amino tridecapeptide portion of the gastrin heptadecapeptide (SHG:1–13) (30). This observation excluded the possibility that cytoplasmic delta cell immunoflorescence was due not to the presence of gastrin but rather to cholecystokinin-pancreozymin. Such observations are equally applicable to the study of the distribution of gastrin cells in the mucosa of the upper small intestine which, unlike the antrum of the stomach, contains CCK-PZ as well as gastrin, thereby providing potential problems in the interpretation of results from immunofluorescence studies using antibodies to gastrin which cross-react, even minimally, with CCK-PZ.

The enormous contributions of Berson and Yalow in the development of radioimmunoassay are evident to all. The questions which they have asked and the answers which they have provided in regard to structural and functional characteristics of gastrin by application of radioimmunoassay techniques are of great value. It is anticipated that radioimmunoassay of gastrin will provide much information to answer important remaining questions concerning the behavior, structure and forms of this most interesting gastrointestinal hormone.

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# Circulating Gastrin in Peptic Ulcer Disease

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Gastrin causes the hypersecretion that produces the ulcers seen in patients with gastrin-producing tumors. The possible role of gastrin in other forms of ılcer disease is currently a popular subject for investigation and speculation.

All of the physiologic mechanisms concerned with stimulation of gastric acid secretion in response to feeding, including the cephalic, gastric, and intestinal phases, involve release of gastrin along with other mediators. Most of our ideas about the role of gastrin in regulating acid secretion come from studies on dogs. There are important differences between man and dog in the physiology of gastrin. For example, antrectomy in man moderately decreases the response to all stimuli, including sham feeding and histamine, but the pre-antrectomy level cannot be restored by giving exogenous gastrin (1, 2). By contrast, antrectomy in the dog greatly decreases the response to sham feeding, but small doses of exogenous gastrin restore the response (3). Antrectomy in dog increases the response of Pavlov pouches to histamine (4) by an unknown mechanism that apparently has no counterpart in man. It is now clear that not all the effects of antrectomy can be explained by removal of gastrin and that extra-antral sites for gastrin production and release exist.

The contribution of gastrin to basal secretion is uncertain. In theory one might expect to find either a positive or negative correlation between serum gastrin and basal secretion—positive if gastrin were driving basal secretion and negative if basal secretion were inhibiting gastrin release. In the event, neither positive nor negative correlation between basal acid output and serum gastrin levels has been found in normal subjects or ulcer patients with normal or increased basal secretion (5). The inverse correlation between basal acid secretion and serum gastrin levels seen in some groups of subjects (6) depends almost entirely on high gastrin levels in some subjects with achlorhydria or marked hypochlorhydria. Apparently only when the decrease in acid secretion is very marked or total is the loss of feedback inhibition of gastrin release by acid sufficient to cause elevation of serum gastrin. This elevation is caused not only by lack of acid inhibition of gastrin release but also by hyperplasia of gastrin cells in the antrum presumably resulting from chronic lack of acid inhibition. Not all achlorhydrics and hypochlorhydrics have elevated serum gastrin; those without elevated serum gastrin have gastritis involving the antrum (7) which presumably prevents hyperplasia of gastrin cells from occurring.

The poor correlation between basal gastrin concentrations measured by radioimmunoassay and basal acid secretion rates is not surprising. In their pioneering work on circulating forms of gastrin, Solomon Berson and Rosalyn

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Yalow demonstrated a larger molecular form of gastrin predominating in the circulation (8, 9). This form was called "big gastrin" and was estimated to have a molecular weight of approximately 7000. Gregory recently has isolated from human Zollinger-Ellison tumor tissue a pair of larger, less acidic gastrins which contain the heptadecapeptide amide as a C-terminal sequence and are linked to another peptide by a lysyl-glutaminyl peptide bond (10). The amino acid composition of the other peptide fragment is $pro_4$, $glu_2$, $gly_2$, $leu_2$, $lys_2$, $ala_1$, $asp_1$, $his_1$, $ser_1$, $val_1$. The whole "big gastrin" molecule contains 34 amino acids. As with the heptadecapeptide gastrins, there appears to be a sulfated and a nonsulfated form. It seems reasonable now to refer to the forms of heptadecapeptide gastrin as G-17 I and G-17 II and the "big gastrins" as G-34 I and G-34 II. Another molecular form of gastrin described by Yalow and Berson (11) elutes from Sephadex columns in the same general area as serum albumin. This was called "big, big gastrin" by them and as yet the amino acid composition of this material has not been determined.

The above discussion indicates that more than one form of gastrin is present in the circulation, probably at least five or six. Rehfeld (12) has described two components in the region of G-34 with apparent differences in molecular weight. Concentrations of serum gastrin as measured by radioimmunoassay reflect the inhibition of binding between labeled antigen and antibody. Differences in potency in the immunoassay among the various gastrins could produce different results when the same serum specimen is tested with antibodies with different spectrums of specificity for these different gastrins. Although Yalow and Rehfeld have reported that trypsin conversion of the large molecular forms to G-17 did not change the immunologic potency in their systems, this has not been determined for other systems. Thus the measurement of basal and stimulated gastrin concentrations potentially is subject to considerable variability among various laboratories.

Another source of possible confusion in correlating circulating gastrin and acid secretion is an apparent difference in acid stimulation produced by equimolar circulating concentrations of G-17 and G-34. Using purified human G-34 provided by Professor Gregory, we have found that it is necessary to infuse G-34 at rates which produce three-fold higher gastrin concentrations as measured by radioimmunoassay to produce equivalent acid secretory responses in the dog when compared with porcine G-17 (Debas, Walsh, and Grossman, unpublished observations). Fortuitously, the longer half-life of G-34 leads to equivalent acid responses when the molar infusion rate of G-17 and G-34 is identical. In other words, the lower biologic potency of G-34 (higher blood level needed for equal response) is counterbalanced by a longer half-life resulting in an apparent equivalence of "potency" of G-34 and G-17 where "potency" is judged on the basis of doses required for equal responses. Thus measurements of circulating gastrin in man reflect the sum of gastrin concentrations of molecular forms with different potencies at the same circulating concentration. Until the distribution of molecular forms in the serum of different individuals has been determined, it is not possible to conclude that there is poor correlation between circulating gastrin and acid secretion in normal subjects and those with peptic ulcer. On the other hand, such a conclusion is justified in achlorhydric subjects with very high concentrations of gastrin.

### *Gastrin Production and Release in the Various Peptic Ulcer Diseases*

*Zollinger-Ellison syndrome.* This syndrome is caused by gastrin hypersecretion by tumors originating in the pancreas or duodenum ("gastrinomas"). The usual results are hypersecretion of hydrochloric acid by the stomach leading to severe peptic ulcer disease, diarrhea or both. Both G-17 and G-34 have been isolated from pancreatic tumors in these patients (10, 13). The G-17 is chemically identical to that isolated from normal human antrum (13).

Radioimmunoassay of fasting plasma or serum from patients with Z-E usually reveals concentrations of immunoreactive gastrin 5 to 10 times or more higher than normal. With the possible exceptions of retained antrum (14) or marked gastric distension due to pyloric obstruction (15), such values in patients with marked acid hypersecretion may be taken as nearly definitive proof of a gastrin-secreting tumor. The absolute numbers obtained depend on the potency of the standard used by the laboratory performing the test, the characteristics of the antibody used, and other technical factors. In our laboratory a fasting gastrin concentration greater than 500 pg/ml is considered highly suggestive and over 1000 pg/ml is considered diagnostic of Z-E syndrome in the nonobstructed patient with acid hypersecretion. This range is in general agreement with several other laboratories (15–21). Solomon Berson and Rosalyn Yalow demonstrated that the majority of this circulating gastrin is in the form of "big gastrin" (8, 9). Therefore the designation "pg/ml" indicates the equivalent concentration of G-17 rather than the actual weight of circulating gastrin. It would be preferable to express concentrations in molar terms, either pmol/l or fmol/ml (fmol = femtamole = $10^{-15}$ mole); 100 fmol/ml = 220 pg/ml porcine gastrin I or 387 pg/ml human "big" gastrin I. Since most gastrin values have been expressed as pg/ml, such values will be reported in the remainder of this paper. Rehfeld and Stadil have confirmed the observations of Yalow and Berson that the majority of circulating gastrin in Z-E patients is of higher molecular weight (22). Both groups reported another component in the serum of higher molecular weight than G-34, called "big, big gastrin" by Yalow and Berson and "component I" by Rehfeld and Stadil. An apparent difference in molecular size of their third components remains to be resolved.

Z-E syndrome is the chief example of hypergastrinemia free from normal physiologic control. Patients with this disease show little or no increase in serum gastrin following the ingestion of a meal. This observation has been useful in some cases as a diagnostic test to differentiate patients with "antral hyperresponsiveness" and acid hypersecretion from those with Z-E syndrome (23). Some patients with documented Z-E syndrome have serum gastrin concentrations which are not diagnostically increased. In some of these patients infusion of calcium may produce an exaggerated rise in serum gastrin and in gastric acid secretion (24, 25) which differs in magnitude from the normal modest responses and may be of diagnostic help. Patients with Z-E also may have an anomalous increase in serum gastrin and in acid secretion during infusion of secretin (14, 26), in contrast to the decrease in these values seen in normal subjects (27). Stimulation tests such as these may offer important additional diagnostic information in patients with borderline serum gastrin values. A special case is the patient with coexisting gastrinoma and parathyroid adenoma. In several such patients marked decreases in serum gastrin have been observed after correction of hypercalcemia. However, diagnostic elevations of serum gastrin have been obtained after intravenous infusion of calcium (24, 25). Another situation in which stimulation tests have been useful is in the differential diagnosis of gastrinomas versus retained antrum. Hansky reported that secretin injection caused a decrease in serum gastrin in such a patient compared with increases found in patients with gastrinoma (14). A recent report suggested that in some cases a syndrome resembling Z-E syndrome may be due to marked hyperplasia of antral gastrin cells (28). In such patients feeding tests will be especially interesting as will radioimmunossay measurements of tissue gastrin content.

*Duodenal ulcer.* In patients with ordinary duodenal ulcer, fasting serum gastrin concentrations have not differed greatly from values obtained in normal subjects. However several studies suggest that the amount of gastrin released in response to a protein meal is greater in duodenal ulcer patients (18, 29–32). It ✓ also has been reported that ulcer patients do not exhibit the normal decrease in gastrin concentrations during the evening and early morning (15). It is possible that the normal feedback mechanism by which low antral pH inhibits antral gastrin release is faulty in duodenal ulcer patients as suggested by Berson and Yalow (33) but this possibility has not been studied systematically. Emas reported that antral gastrin content measured by bioassay was similar in normal subjects and in those with duodenal ulcer (34). Patients with gastric ulcer had lower concentrations of antral gastrin. Similar studies have not been reported with immunoassay or immunohistologic techniques.

Patients with impaired gastric emptying may have elevation of serum gastrin with reversion to normal during treatment with nasogastric suction (15).

Gastrin radioimmunoassay also has been applied to patients with duodenal ulcer who have been treated surgically. There has been general agreement that truncal (31, 35, 36) or selective gastric vagotomy (37, 38) results in increased concentrations of fasting gastrin and in exaggerated increases after a meal. There is some disagreement as to whether gastrin response to insulin is abolished (39) or only diminished (40). Antrectomy results in some diminution of basal gastrin concentrations but usually not to unmeasurable levels (8, 35). The gastrin stimulation seen after feeding is greatly diminished in patients with antrectomy and vagotomy when the gastric remnant is anastomosed to the jejunum but near normal postprandial increases are often found when gastroduodenal anastomosis is performed (31). The latter response probably reflects release of duodenal gastrin. By bioassay and by radioimmunoassay the human duodenum contains from one-third to nearly equal amounts of gastrin compared to the gastric antrum (34, 41, 42).

Patients with recurrent ulcer after gastric surgery present problems of differentiating inadequate surgical procedure from gastrinoma. There have been few reports of circulating gastrin levels in such patients. We have found in patients with vagotomy alone that those with "complete" vagotomy as judged by absence of acid response to insulin tend to have higher gastrins than those with "incomplete" vagotomy although both groups are higher than prevagotomy. There is no extensive study of serum gastrin in patients with recurrence after antrectomy. There is one case report of increased gastrin in a patient with isolated retained antrum (14). This condition seems to be rare in the United States. Our impression is that unless they have gastrinomas most patients with recurrent ulcer after surgery do not have gastrin values that differ markedly from those of patients without recurrent ulcer.

*Gastric ulcer.* There is no evidence that gastrin plays an important role in the pathogenesis of gastric ulcer. Emas (34) found that antral gastrin concentration in these patients was lower than in those with duodenal ulcer or in normal subjects. Most patients with gastric ulcer have antral gastritis which may impair gastrin formation and release. On the other hand they tend to have diminished acid secretion which favors release of gastrin and hyperplasia of gastrin cells. The balance between the depressive effect of gastritis and the augmentory effect of hypochlorhydria probably varies from patient to patient. McGuigan (43) reported that circulating gastrin was about twice as high in gastric ulcer as in duodenal ulcer or normal subjects and that the increase in blood gastrin was correlated with decreased acid secretion. Dragstedt (44) hypothesized that gastric stasis stimulated gastrin release that caused hypersecretion leading to gastric ulcer. Since the elevated serum gastrin levels seen in some patients with gastric ulcer are associated with decreased acid secretion, Dragstedt's hypothesis is not supported.

### *Diagnostic Usefulness of Radioimmunoassay of Gastrin in Peptic Ulcer Disease*

Presently the only established diagnostic use of radioimmunoassay of gastrin is for evaluating the possibility of gastrinoma. Since the introduction of this measurement a number of patients with gastrinoma have been recognized who did not have the flagrant form of the syndrome and, indeed, whose disease resembled ordinary duodenal ulcer disease (45). But with this dividend of extra diagnostic power came the corresponding cost of borderline cases with uncertain diagnosis. Further development of stimulation and suppression tests promises to improve the distinction between gastrinoma and other forms of ulcer disease. Radioimmunoassay probably will be useful also in the diagnosis of isolated retained antrum, apparently now a rare condition. It is possible that differences will be found between duodenal ulcer and normal subjects in the distribution of the various molecular forms of gastrin in tissue and in the circulation but this has not yet been studied. The clinical applicability of basic science knowledge often comes from unexpected quarters, so we should not strain to predict the future but rest content that Solomon Berson's contributions will endure.

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# Observations on the Control and Release of Growth Hormone in Plasma

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Knowledge of the regulation of the synthesis and release of growth hormone (GH) has been accumulated slowly in contrast to the information available on other pituitary hormones. The relative difficulty, nonspecificity, and insensitivity of the bioassay methods that have been used in these studies have delayed the acquisition of definitive data. The development of sensitive immunoassay techniques by Hunter and Greenwood (1) and by Glick and his associates (2) has contributed enormously to more recent studies of the regulation of GH secretion, but many problems still remain to be clarified. A comprehensive review of the subject was made by Reichlin in 1966 (3), and subsequent observations will be summarized in this manuscript, particularly as they relate to the control of release of GH in the rat.

Radioimmunoassay has demonstrated that plasma GH is subject to wide and rapid fluctuations. Elevated levels are seen during insulin- or tolbutamide-induced hypoglycemia (4–8), during falling blood glucose levels seen after a glucose load or postprandially, during fasting (9, 10), exercise (5, 11–13), intravenous amino acid infusions (14–16), operations (17–19), and after the administration of pyrogen (10, 20–23). The effects of exposure to cold on plasma GH remain unclear (24, 25). Peaks in plasma GH occur most commonly during deep sleep, with smaller peaks occurring in subsequent sleep phases (25–30). In the monkey, minor trauma, spontaneous excitement, pain, noise, apprehension, intravenous injection of histamine, epinephrine, pitressin and vasopressin, hemorrhage, and arousal from nembutal anesthesia are associated with abrupt increases in GH secretion. These stimuli are comparable to those which have long been known to increase ACTH release (31, 32).

Other hormones modulate the GH response to insulin hypoglycemia and arginine infusion. Estrogens enhance the response to arginine and to exercise (33–35). Androgens greatly enhance the plasma GH response to hypoglycemia and arginine infusion (36). A potent progestational agent, 6 methyl-17$\alpha$-hydroxyprogesterone acetate, suppresses the response both to insulin and arginine (37). Suppression of GH release by corticosteroids (7, 38) is well documented, while ACTH is much less effective than corticosteroid administration (39). Diminished responsiveness is seen in both hypo- and hyperthyroidism (40). Large doses of vasopressin and epinephrine increase plasma GH levels in the absence of other provocative stimuli (25).

Much of the early work on the role of the central nervous system in the

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regulation of GH secretion was carried out in the rat. Del Vecchio and associates (41) were the first to suggest that crude posterior pituitary extracts could increase rat epiphyseal cartilage width, an observation later confirmed by Hiroshige and Itoh (42). Since hypophysectomy prevented this effect, a growth hormone-releasing factor (GHRF) analogous to thyrotropin releasing factor was postulated. Reichlin's observations (43, 44) also suggested that hypothalamic control existed over GH production. Hypothalamic extract stimulated GH release in vitro, and repeated administration of the extract widened tibial epiphyseal cartilages and increased body weight in intact but not in hypophysectomized rats. More convincing results were obtained by Deuben and Meites (45) in in vitro studies using tissue culture of rat anterior pituitaries and rat hypothalamic extracts. These observations were confirmed by Schally and his associates (46) on bovine and porcine hypothalamic extracts. Equivocal evidence for the existence in the dog of a hypothalamic neurohumoral factor regulating GH secretion was reported by Franz and associates (47). Pecile and associates (48) in search of an assay for GHRF reported that intracarotid or intravenous injection of a crude hypothalamic extract in rats resulted in a profound fall of pituitary GH content compared with injections of control tissues. The GH content of these pituitaries was bioassayed by the tibia test procedure of Greenspan and associates (49). Since these observations were made, an intensive search for growth hormone releasing factors has been carried out. Crude hypothalamic extracts from guinea pig (50), rat (51), cow (47), dog (52), sheep (53), monkey and man (54) have been claimed to deplete pituitary GH content in vivo or to release pituitary GH in vitro. Attempts to obtain GHRF in purer form have been made using gel filtration and ion exchange chromatography of crude preparations of bee, sheep, and pig hypothalamic (55–58). At present, most in vivo evidence rests on the measurement of GH secretion by the depletion of pituitary GH using the tibia test bioassay, although we (59) and others have stressed the need for caution in interpreting the results obtained with this method. More recently, Krulich and associates (60) have claimed that a factor inhibiting the release of GH has been isolated from sheep and rat hypothalamic extract.

The hypothalamus would also appear to play an important role in the regulation of growth hormone secretion in primates. Patients with pituitary stalk sections become unresponsive to stimuli that increase GH secretion (4). Specific hypothalamic lesions in the monkey can prevent insulin-induced GH secretion (61). More recently, the effects of certain hypothalamic extracts on the plasma levels of GH have been reported (62–64), although major controversy exists as to the specificity of this response (65, 66). These data are in keeping with earlier observations, summarized and contributed to by Reichlin (44) on the effects of experimental brain lesions on growth. To be effective, such lesions must involve the anterior median eminence, including a portion of the primary plexus. Additional convincing data from stimulation experiments have been reported by Frohman and associates (67, 68). They found that both destruction and stimulation of the ventro-medial hypothalamic nucleus in rats resulted in the predicted changes in plasma and pituitary GH content as measured by immunoassay. Although hypothalamic control of GH release is now virtually undisputed, the identification and synthesis of substances with growth hormone-releasing activity in both bioassay and radioimmunoassay systems has remained elusive.

The influence of catecholamines on secretion of GH has been difficult to evaluate, although evidence of a relationship between them is increasingly convincing. Infusions of epinephrine in humans did not alter plasma levels of GH, but the simultaneous administration of propranolol unmasked a stimulatory effect of epinephrine (69, 70). However, intravenous infusions of epinephrine in sheep inhibited the GH response to arginine, and this was not influenced by phentolamine or propranolol (71, 72).

Hypothalamic extracts or prostaglandin $E_1$ can stimulate adenyl cyclase activity and increase cyclic AMP concentration in the rat anterior pituitary tissue in vitro (73). Furthermore, hypothalamic extracts, prostaglandin $E_1$, or dibutyryl cyclic AMP can release GH from the isolated rat pituitary gland (74, 75). The major brain catecholamines, norepinephrine and dopamine, are found in high concentrations in the hypothalamus and median eminence (76, 77). Catecholamines are known to influence adenyl cyclase in many nonpituitary tissues (78), although their effects on adenyl cyclase in pituitary tissue are not clear. Direct effects of adrenergic agents on GH release from pituitary tissue in vitro have not been demonstrated, but there is evidence that hypothalamic catecholamines influence depletion of GH from the anterior pituitary gland in rats (79).

Direct infusions of catecholamines into the lateral ventricles of the rat induced a depletion of GH activity in the pituitary (80). Norepinephrine and dopamine induced disappearance of GHRF activity from the hypothalamus and its appearance in the plasma of hypophysectomized rats (81). In intact rats, norepinephrine (0.05–0.0025 $\mu$g) given intraventricularly resulted in a depletion of pituitary GH activity, but dopamine was ineffective. Recently, microinjection of norepinephrine into the posterior basal midhypothalamus was found to stimulate GH release in the conscious baboon (82). These results suggest a hypothalamic site of action for the growth hormone-releasing effect of catecholamines and that norepinephrine is the synaptic transmitter that facilitates GHRF release from the neurosecretory neurons.

Oral administration of L-dopa has been shown to increase plasma GH levels in patients with Parkinson's disease (83). Either oral or intravenous administration of the drug to normal subjects also induces this effect on GH secretion. Glucose administration does not block the effect, but it is minimally inhibited by phentolamine (84).

Studies with adrenergic blocking agents in man and the baboon have demonstrated depressed GH secretion after $\alpha$-adrenergic blockade and increased GH secretion after $\beta$-blockade (85–88). A stimulatory effect of propranolol was inhibited by isoproterenol or oral glucose administration. Alpha stimulating agents, such as methoxamin and phenylephrine, increased plasma GH levels in the human, and this effect was blocked by phentolamine. Oral administration of propranolol has been shown to augment the plasma GH response to glucagon in man (89).

Since depletion of pituitary GH may not correlate closely with changes in blood levels of GH (90, 91) and since an effect of cyclic AMP on rat GH secretion has never been clearly demonstrated in vivo, we sought means to examine the effects of dibutyryl cyclic AMP, prostaglandin $E_1$, and adrenergic agents on plasma GH in anesthetized rats. It was found that the urethane-anesthetized animal was suitable for the purpose, exhibiting low and stable baseline values for immunoreactive GH with consistent responses to certain stimuli (92). It was found that adrenergic effects on GH secretion in rats differ from those in man (87). The study was therefore extended to an examination of effects of vasopressin, chlorpromazine, methyl amphetamine, and L-Dopa, which have been shown to affect GH secretion in man (83, 93–95). The pattern of effects of these agents on rat GH also differed from that on human GH. In addition, the effect of synthetic GHRF (L-621,117-00F07) (L-622,443-00X02) supplied by the Department of Protein Chemistry of the Merck Institute (Rahway, New Jersey), having the amino acid sequence of a peptide with GH-releasing activity isolated from porcine hypothalamus reported by Schally and his associates (96) on GH release in the animal model, was tested. The results of these experiments help clarify some of the hypothalamic mechanisms regulating GH secretion in different species.

## Materials and Methods

Male Sprague-Dawley rats (Canadian Breeding Farm, Ste-Catherine, Quebec) weighing 180–220 g were used. Rats were housed for 1 week before use in a temperature controlled, airconditioned room under artificial lighting (lights on at 0700, off at 2100 hr), and were maintained on Purina® laboratory chow and water ad lib.

### *Experimental procedures*

After overnight fasting (16 hr), animals were anesthetized with urethane (150 mg/100 g of body wt, ip). Thirty minutes later, test substances were injected into one jugular vein in a volume of 0.1 ml per 100 g body wt in groups of four to six rats.

### *Test drugs*

The following drugs were employed: dibutyryl cyclic AMP ($N_6$, $O_2$-dibutyryl adenosine-3; 5′-cyclic monophosphoric acid); prostaglandin $E_1$; dl-isoproterenol HCl; dl-propranolol HCl; l-phenylephrine HCl; phentolamine; synthetic arginine vasopressin; synthetic lysine vasopressin; histamine phosphate; formalin; crystalline insulin; chlorpromazine hydrochloride, methylamphetamine hydrochloride, L-Dopa (L-β-3,4-Dihydroxyphenylalanine) dl-propranolol HCl and synthetic GHRF. All drugs except prostaglandin $E_1$ and L-Dopa were dissolved in physiological saline solution. Prostaglandin $E_1$ was dissolved in 0.2 M phosphate buffer (pH 7.2) at a concentration of 200 $\mu$g/ml and diluted with physiological saline (97). L-Dopa was first dissolved in a warm solution of 0.5 N HCl to which 0.5 N NaOH was added to bring pH to 2.8 (98). Two decapeptides L-621,17-00F07 and L-622,443-00X02 supplied by Dr. Ralph F. Hirschmann, Merck, Sharp and Dohme Research Laboratories, were given. L-621,117 has the amino acids sequence of a peptide with GH-releasing activity reported by Schalley et al (96). L-622,443 contains a glutamine residue in position 9 in place of the glutamic acid residue, and thus represents the amino acid sequence of the amino-terminal decapeptide of the $\beta$ chain of porcine hemoglobin. The rationale for the synthesis of the latter compound is described by Veber et al (99).

### *Radioimmunoassay of rat growth hormone*

Plasma rat GH measurements were performed in duplicate on 0.1 ml aliquots of plasma using a specific radioimmunoassay (92). Monkey anti-rat GH serum (NIAMD-Rat GHS-1) and a rat GH reference preparation (NIAMD-Rat GH-RP-1) were kindly supplied by Dr. A. F. Parlow through the NIAMDD Pituitary Hormone Distribution Program, Bethesda, Md. Porcine GH (P522A) supplied by Dr. A. E. Wilhelmi was used for iodination with $^{125}$I (100). Separation of bound from free hormone was effected by the talcum method

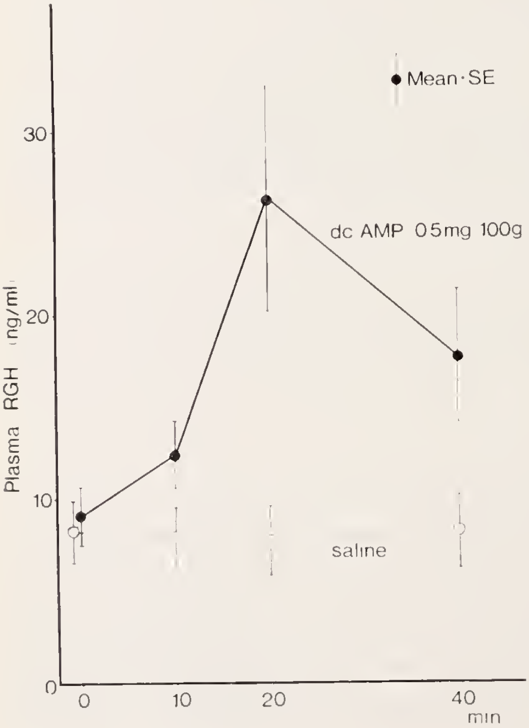

FIG. 1. Effect of dibutyryl cyclic AMP on plasma rat GH (RGH).

(101). The minimal detectable quantity of plasma rat GH was 2.5 ng/ml. Repeated determination of one pooled plasma on 10 different days gave a coefficient of variation of 9.3 percent. Student's t-test was used to determine significance of differences in plasma rat GH values between groups.

## Results

### *Effect of dibutyryl cyclic AMP on plasma rat GH (Fig. 1)*

Plasma rat GH rose significantly after the injection of dibutyryl cyclic AMP (0.5 mg/100 g, body wt) ($P < 0.01$). The mean plasma GH level 20 min after the injection was 26.5 ng/ml, ± standard error 7.0 ng/ml. No significant change was observed in plasma rat GH in the control group, in which physiological saline solution was injected.

### *Effect of prostaglandin $E_1$ on plasma rat GH (Fig. 2)*

The intravenous administration of prostaglandin $E_1$ (5 $\mu$g/100 g body wt) caused an increase in plasma rat GH 10 min after injection ($P < 0.05$ by comparison with control group). This rise was not affected by the simultaneous administration of propranolol (400 $\mu$g/100 g body wt), a beta adrenergic blocking agent (Fig. 3).

### *Effects of adrenergic stimulating or blocking agents on plasma rat GH*

The intravenous injection of isoproterenol (10 $\mu$g/100 g body wt), a beta adrenergic stimulating agent, provoked a rise in plasma GH 10 min after the injection (Fig. 4) ($P < 0.05$). Injection of propranolol (400 $\mu$g/100 g body wt), a beta adrenergic blocking agent, tended to lower plasma rat GH levels. The

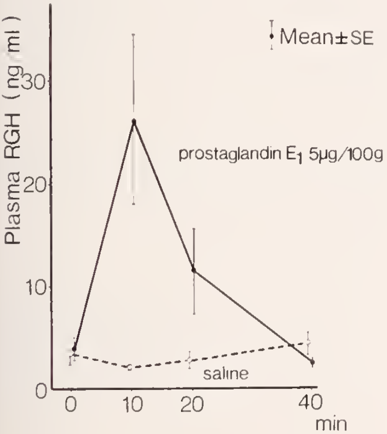

FIG 2.

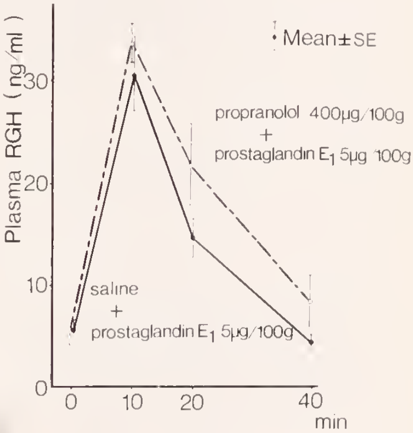

FIG. 3.

FIG. 2. Effect of prostaglandin E1 on plasma rat GH.
FIG. 3. Effect of prostaglandin E1 alone or with propranolol on plasma rat GH.

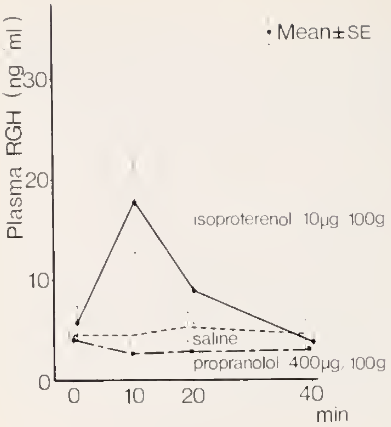

FIG. 4.

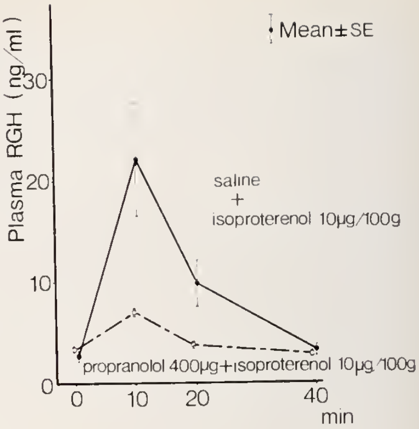

FIG. 5.

FIG. 4. Effect of isoproterenol or propranolol on plasma rat GH.
FIG. 5. Effect of isoproterenol alone or with propranolol on plasma rat GH.

concomitant administration of propranolol with isoproterenol significantly suppressed the plasma rat GH response to isoproterenol, as shown in Fig. 5 ($P < 0.05$).

The intravenous injection of phentolamine (100 μg/100 g body wt), an alpha adrenergic blocking agent, elicited a rise in plasma rat GH (Fig. 6) ($P < 0.05$). On the other hand, plasma GH levels tended to fall after the injection of phenylephrine (30 μg/100 g body wt), an alpha adrenergic stimulating agent. Injection of phentolamine together with phenylephrine suppressed the plasma rat GH response to phenylephrine (Fig. 7) ($P < 0.05$).

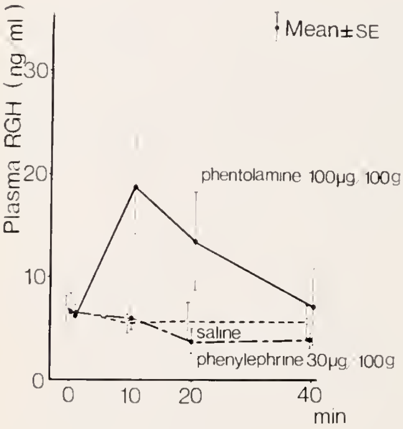

FIG. 6.

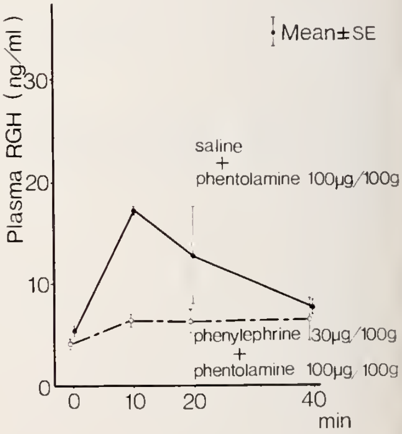

FIG. 7.

FIG. 6. Effect of phentolamine or phenylephrine on plasma rat GH.
FIG. 7. Effect of phentolamine alone or with phenylephrine on plasma rat GH.

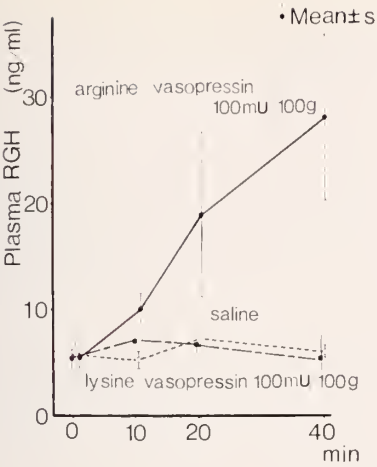

FIG. 8.

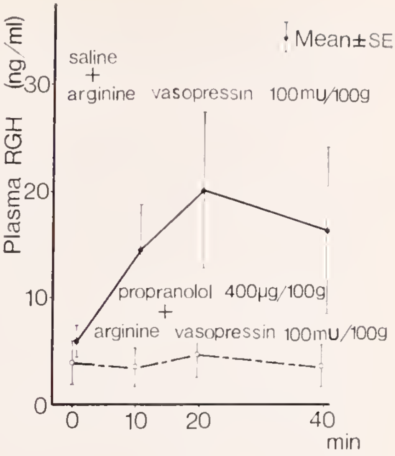

FIG. 9.

FIG. 8. Effect of synthetic arginine vasopressin or synthetic lysine vasopressin on plasma rat GH.

FIG. 9. Effect of synthetic arginine vasopressin alone or with propranolol on plasma rat GH.

### *Effect of vasopressin on plasma rat GH*

The intravenous injection of synthetic arginine vasopressin (100 mU/100 g body wt) caused a significant rise in plasma rat GH (Fig. 8) ($P < 0.05$). After the administration of synthetic lysine vasopressin (100 mU/100 g body wt), however, there was no significant change in plasma rat GH. The plasma GH response to arginine vasopressin was completely abolished by simultaneous administration of propranolol, as shown in Fig. 9.

### *Effects of various stressful agents on plasma rat GH*

As shown in Table I, the following stimuli failed to cause a significant increase in plasma rat GH; administration of histamine (100 μg/100 g body

TABLE I

*Effects of Various Stressful Agents on Plasma Rat GH*

| Treatment | Plasma rat GH (ng/ml) | | | |
|---|---|---|---|---|
| | Pretreatment | 10 min | 20 min | 40 min |
| Saline (4) | 4.0 ± 0.3* | 4.3 ± 0.7 | 4.5 ± 1.1 | 4.9 ± 0.9 |
| Histamine 100 μg/100 g (5) | 6.1 ± 1.0 | 7.1 ± 1.6 | 6.9 ± 2.0 | 5.4 ± 1.5 |
| Formalin 10% 0.1 ml/100 g (4) | 4.6 ± 0.3 | 6.0 ± 0.9 | 4.8 ± 0.3 | 4.6 ± 0.4 |
| Laparotomy (4) | 7.0 ± 1.8 | 5.9 ± 1.8 | 4.5 ± 1.1 | 3.9 ± 1.4 |
| Saline (4) | 6.0 ± 0.9 | 8.4 ± 1.6 | 8.0 ± 1.4 | 5.5 ± 0.6 |
| Insulin 0.2 U/100 g (4) | 3.4 ± 0.5 | 6.5 ± 1.5 | 6.4 ± 1.6 | 4.3 ± 0.9 |

Number of rats in parenthesis.
* Mean ± SE.

wt), administration of formalin (10%, 0.1 ml/100 g body wt); laparotomy with exposure of abdominal organs; and induction of hypoglycemia with insulin (0.2 U/100 g body wt).

*Effect of chlorpromazine on plasma rat GH (Fig. 10)*

Plasma rat GH rose 20 to 40 min after the intravenous injection of varying doses of chlorpromazine (50 $\mu$g, 100 $\mu$g/100 g body wt) ($P < 0.05$, $P < 0.01$, $P < 0.05$, respectively, compared with the saline control group). This response was dose-related and the minimum effective dose of chlorpromazine was 50 $\mu$g/100 g body wt.

As shown in Fig. 11, the rise in plasma GH induced by chlorpromazine (50 $\mu$g/100 g body wt) was inhibited by simultaneous administration of L-Dopa (1 mg/100 g body wt) ($P < 0.05$), compared with the control group in which pH 2.8 solution (vehicle) was injected in a volume of 0.1 ml/100 g body wt immediately before the administration of chlorpromazine.

*Effect of L-Dopa on plasma rat GH (Fig. 12)*

The intravenous injection of L-Dopa alone (1 mg/100 g body wt) caused no significant change in plasma rat GH. A higher dose of L-Dopa (5 mg/100 g body wt) tended to decrease plasma rat GH levels, but this effect was not statistically significant. A single intravenous injection of pH 2.8 solution (vehicle) had no effect on plasma GH levels.

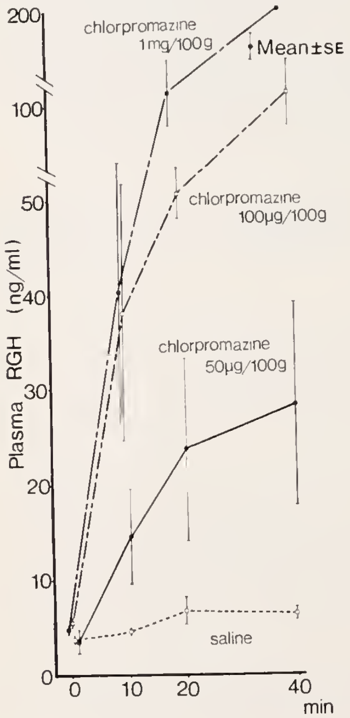

FIG. 10. Effect of chlorpromazine at the doses indicated on plasma rat GH.

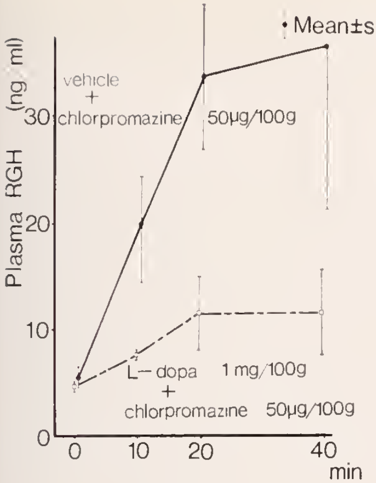

Fig. 11.

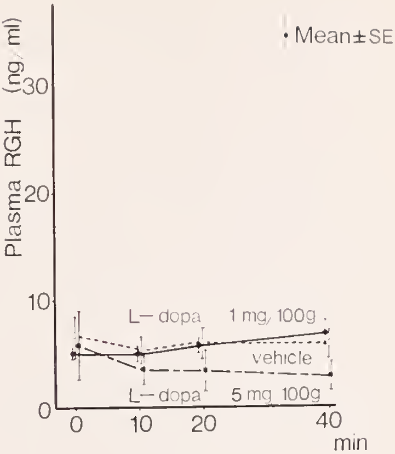

Fig. 12.

Fig. 11. Effect of chlorpromazine alone or with L-Dopa on plasma rat GH.
Fig. 12. Effect of L-Dopa on plasma rat GH.

*Effect of methylamphetamine on plasma GH (Fig. 13, 14)*

The intravenous injection of methylamphetamine (0.5 mg/100 g body wt) caused a significant increase in plasma rat GH 40 min after the injection in all rats examined (P < 0.05). This response was partly but significantly suppressed by concomitant administration of L-Dopa (2 mg/100 g body wt) (P < 0.05), compared with the control group in which methylamphetamine was injected immediately after the intravenous injection of pH 2.8 solution (vehicle)

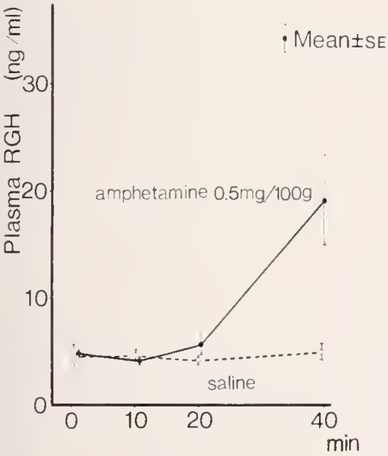

Fig. 13.

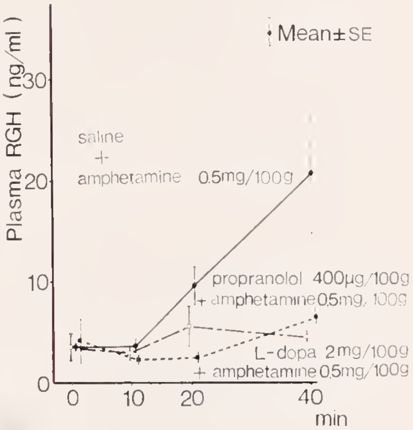

Fig. 14.

Fig. 13. Effect of methylamphetamine on plasma rat GH.
Fig. 14. Effect of methylamphetamine alone or with L-Dopa or propranolol on plasma rat GH.

instead of L-Dopa. The simultaneous administration of propranolol (400 μg/100 g body wt), a beta adrenergic blocking agent, caused a significant inhibition of the plasma rat GH response to methylamphetamine.

*Effect of synthetic GHRF on plasma rat GH*

The intravenous injection of varying doses of synthetic GHRF (L-621,117-0.2 μg, 2 μg, 20 μg/100 g body wt) appeared to influence GH release. In these experiments plasma rat GH did fluctuate spontaneously but there appeared a possibility that the decapeptide injected at the intermediate dose had GH-releasing activity for, although the peak response at 20 min was not significantly different from the control group ($P > 0.2$ $P < 0.05$ $P > 0.02$), it was significantly different from its own preinjection level (Fig. 15).

These experiments were repeated in an identical fashion by another worker whose results are listed in Figure 16, and it is seen that there was no significant elevation of plasma rat GH in any group.

At this time an opportunity arose for the original observer to repeat these experiments and on this occasion two dose levels of the decapeptides were tested (2 μg and 10 μg/100 gm body wt). The results obtained are summarized in Fig. 17.

The marginal elevation of plasma rat GH in the group receiving 2 μg decapeptide/100 gm body weight was not significantly different either from the control group or from its own preinjection level.

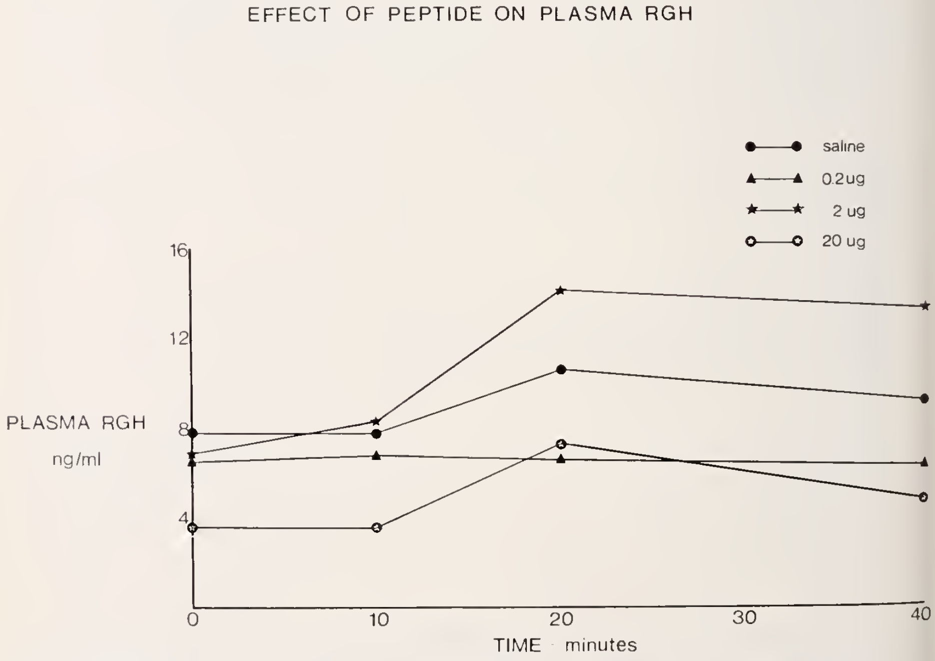

Fig. 15. Effect of synthetic GHRF (L-621,117-00F07) as doses indicated on plasma rat GH.

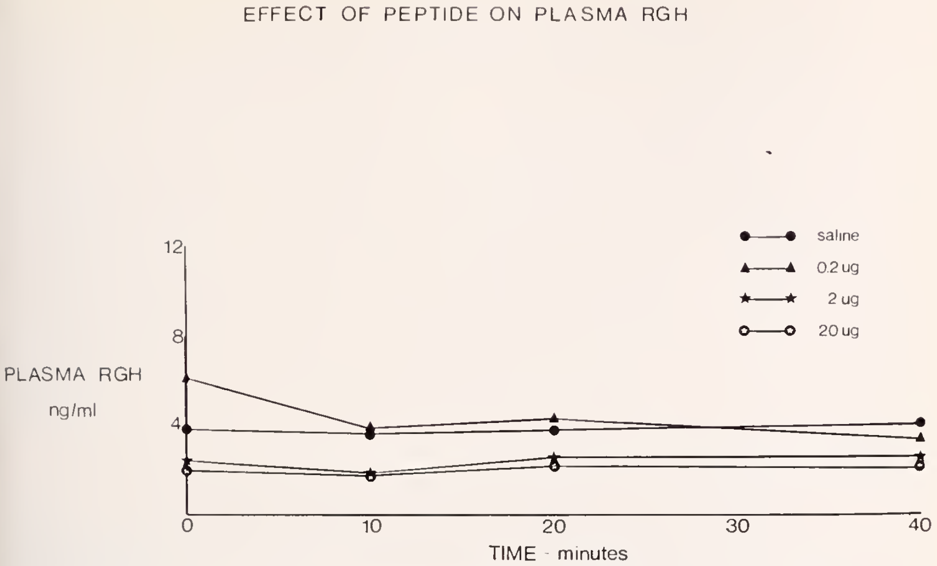

FIG. 16. Effect of synthetic GHRF at identical doses to Fig. 15 on plasma rat GH.

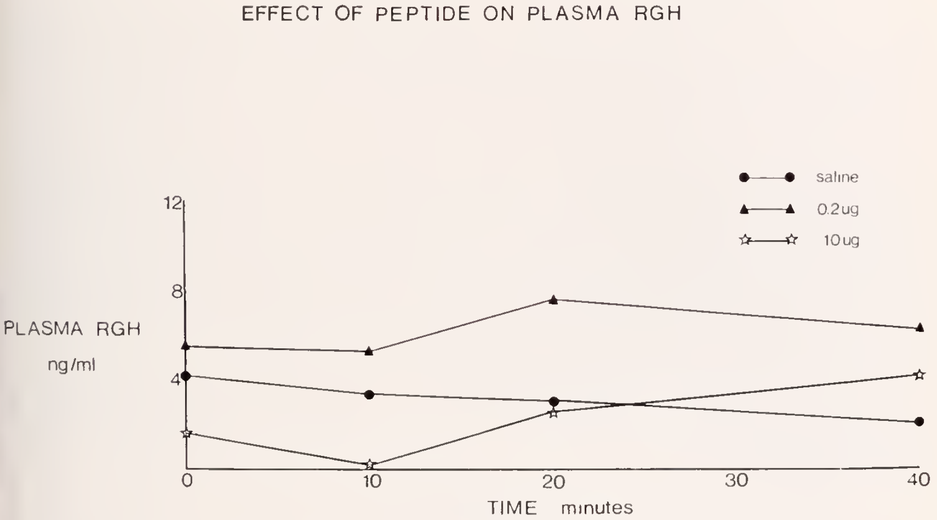

FIG. 17. Effect of synthetic GHRF at doses indicated on plasma rat GH.

As the possibility of earlier response had not been explored in detail, the experiments were repeated with more frequent time intervals; the results are shown in Fig. 18. These data indicated that an early response had not been overlooked. It was of interest that the elevation of plasma rat GH 20 minutes after injection in the group receiving 2 $\mu$g decapeptide/100 gm body weight fell just short of statistical difference over its own preinjection level ($0.1 > P > 0.05$). However, it was recognized that hypovolemia per se resulting from the

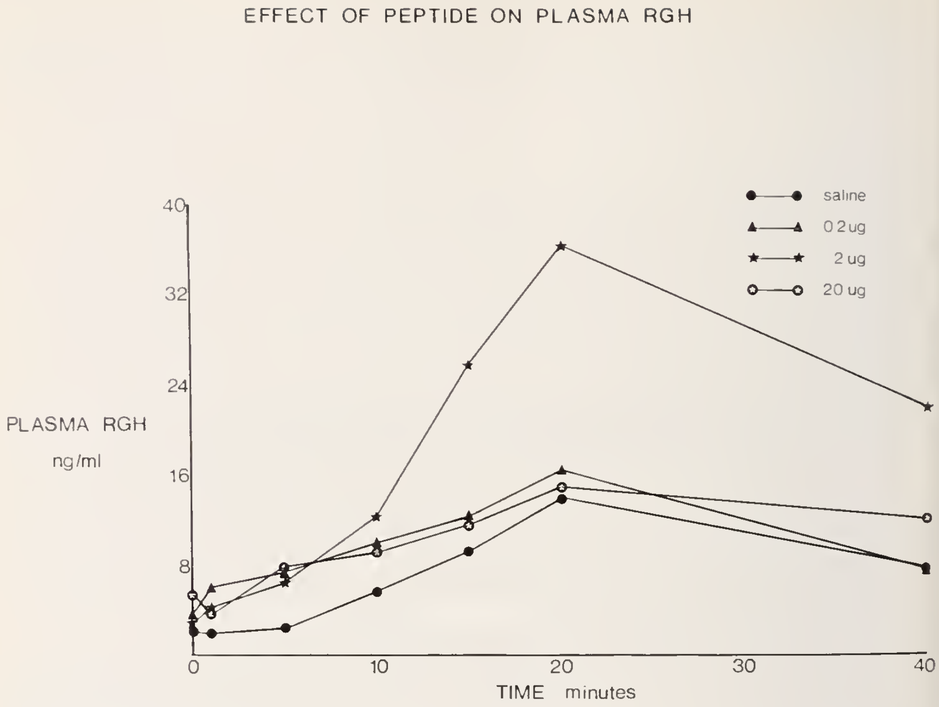

Fig. 18. Effect of synthetic GHRF on plasma rat GH—venous blood sampled at early and late postinjection intervals.

frequent blood sampling might have played a causative role. It was therefore considered worthwhile to focus efforts on this intermediate dose level of decapeptide with due regard to avoidance of excessive depletion of blood volume. The results of this final experiment, summarized in Figure 19, confirmed our suspicion that the elevation of plasma rat GH observed previously was most likely an artifact resulting from the stress of hypovolemia.

The synthetic peptide (1-622,443-00X02) was tested in an identical model and data comparable to those described above were obtained, not justifying further work in this area.

## Discussion

Plasma rat GH responses to various stimuli were studied under urethane anesthesia (150 mg/100 g body wt). Intravenous injections of dibutyryl cyclic AMP (0.5 mg/100 g body wt) or prostaglandin $E_1$ (5 $\mu$g/100 g body wt) caused significant increments in plasma rat GH. Increments in plasma rat GH were also caused by isoproterenol (10 $\mu$g/100 g body wt) or phentolamine (100 $\mu$g/100 g body wt), and these effects were suppressed by concurrent administration of propranolol or phenylephrine, respectively. Synthetic arginine vasopressin (100 mU/100 g body wt) significantly increased plasma rat GH, and this effect was inhibited by the simultaneous administration of propranolol.

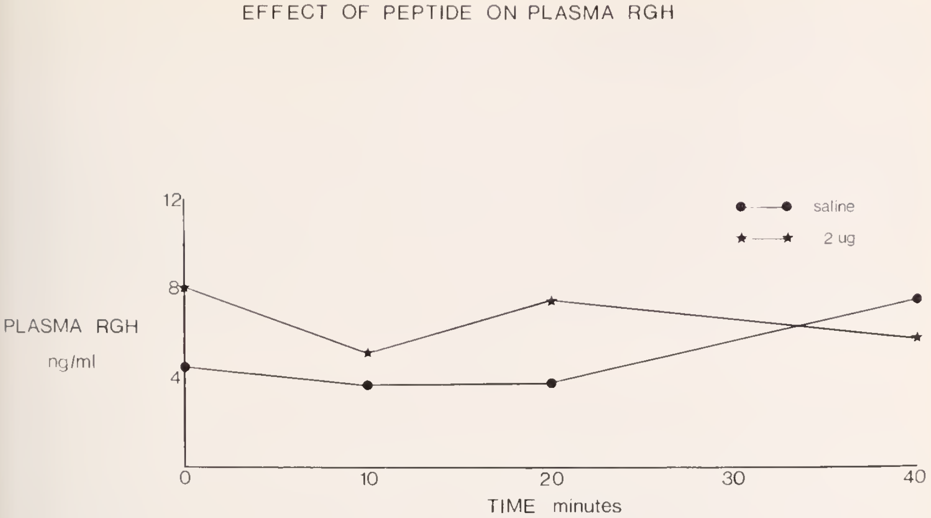

FIG. 19. Effect of intermediate dose of synthetic GHRF on plasma rat GH avoiding excessive blood volume depletion.

Intravenous injections of chlorpromazine (50 μg to 1 mg/100 g body wt) produced marked dose-related increments in plasma growth hormone in these studies. The peak response appeared 20 to 40 min after injection of chlorpromazine. This effect was partially inhibited by simultaneous injection of L-Dopa (1 mg/100 g body wt), though L-Dopa (1 to 5 mg/100 g body wt) alone caused no significant change in plasma rat GH.

Increases in plasma rat GH were also observed after intravenous injection of methylamphetamine (0.5 mg/100 g body wt). This increase was diminished by the concomitant administration either of L-Dopa (2 mg/100 g body wt) or propranolol (400 μg/100 g body wt). The intravenous injection of varying doses of synthetic GHRF caused no significant changes in plasma rat GH.

Our studies in the urethane anesthetized rats have documented changes in plasma GH levels in response to a variety of agents, and have demonstrated inverted effects of alpha adrenergic and beta adrenergic agents by comparison with the effects of these agents in man. The results also show that reciprocal effects of some other stimuli in the two species, as with stresses, chlorpromazine, and L-Dopa may be related to the characteristic effects of alpha and beta adrenergic agents in these species. The common actions of cyclic nucleotides in the two species would be expected by virtue of the messenger hypothesis, but the levels or sites of these actions remain to be established. As suggested, prostaglandin $E_1$ probably acts directly upon the pituitary cells independently of adrenergic receptors. In these experiments in the rat, the stimulator effects of arginine vasopressin and methyl amphetamine clearly depended on beta-adrenergic activity, but levels of these actions are unidentified, and the effects of adrenergic blocking agents on the responses to vasopressin or amphetamines in man remain to be established. It seems clear that the adrenergic mechanism was involved in GH secretion in rats with effects of alpha and beta adrenergic stimuli being at variance with those observed in man.

### Acknowledgments

Prostaglandin $E_1$ was kindly supplied by Dr. J. E. Pike of the Upjohn Company, Kalamazoo, Mich. Phentolamine (Rogitidine®) was purchased from Ciba Co. Ltd., Dorval, Quebec. Chlorpromazine hydrochloride (Elmarine®) and methylamphetamine hydrochloride (Methedrine®) were obtained from Elliott/Marion Co. Ltd., and Burroughs Wellcome & Co. (Canada) Ltd., Montreal, Quebec, respectively. Synthetic GHRF was kindly supplied by Dr. R. F. Hirschmann of Merck Sharp & Dohme Research Laboratories, Rahway, N.J. All other drugs were purchased from Sigma Chemical Company, St. Louis, Mo.

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# Regulation of Growth Hormone and Prolactin Secretion in Patients with Acromegaly and/or Excessive Prolactin Secretion

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## Introduction

Since the application of the radioimmunoassay concept to the measurement of growth hormone (GH) in human serum in 1962 (1), nearly sixty patients with acromegaly have been studied in this laboratory. The fifty patients who underwent, at a minimum, an oral glucose tolerance test with measurement of growth hormone levels, form the basis of the concept of regulated, though excessive, growth hormone secretion in acromegaly as elaborated in the following paragraphs.

The development of a radioimmunoassay system for the measurement of human serum prolactin (2), a decade after the GH assay, has permitted study of the prolactin secretory patterns in women, some of whom exhibit galactorrhea, with high circulating levels of prolactin.

### *Regulation of Growth Hormone Secretion in Acromegaly*

*Oral glucose tolerance tests.* Serum GH responses to the ingestion of 100 g of glucose in 50 patients with acromegaly are summarized in Table I. Baseline values in these 50 patients ranged from 5 to 1800 ng/ml with a median value of 50 ng/ml.

No change in serum GH concentrations followed glucose ingestion in 31 patients. However, suppression of the serum GH level, a qualitatively normal response (3), occurred in 12 acromegalic patients. The suppressive response of serum GH to the ingestion of glucose was consistent in a given patient when restudied. For example, the serum GH levels in patient A. Sa. fell from 160 to 80 ng/ml and 120 to 65 ng/ml following glucose ingestion on two separate occasions.

Failure of the serum GH concentration to suppress into the normal range following oral glucose has been considered a criterion for the diagnosis of

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TABLE I

*Serum GH Response to Oral Glucose, Intravenous Arginine and Insulin-Induced Hypoglycemia in Acromegalic Patients*

| | Number of Patients | Serum Growth Hormone Responses | | |
|---|---|---|---|---|
| | | No change | Decrease* | Increase† |
| Oral Glucose Tolerance Tests | 50 | 31 (62%) | 12 (24%) | 7 (14%) |
| Arginine Infusion | 33 | 18 (55%) | — | 15 (45%) |
| Insulin-Induced Hypoglycemia | 20 | 8 (40%) | — | 12 (60%) |

* Decrease to <50% of baseline.
† Increase to >150% of baseline.

acromegaly (3, 4). In this series of 50 acromegalic patients, two suppressed to less than 5 ng/ml. From baseline values of 13 and 10 ng/ml these patients suppressed to 3 ng/ml following glucose ingestion. Of the remaining 48 patients, none suppressed to less than 15 ng/ml.

Paradoxical stimulation of GH secretion following glucose ingestion occurred in seven of fifty acromegalic patients. In five patients the glucose-induced increment in serum GH ranged from two- to five-fold. This pattern was also consistent when a given patient was studied repetitively. For example, in patient M.G., the GH level rose from 25 to 130 ng/ml, 20 to 72 ng/ml and 12 to 100 ng/ml during three separate glucose tolerance tests. Paradoxical GH secretion in response to ingested glucose is not unique to acromegaly. It has been described in patients with tumors encroaching upon the hypothalamus (5) and in patients with a variety of disorders without recognized structural lesions of the hypothalamus or pituitary (6).

*Arginine and insulin provocative tests.* As listed in Table I, serum GH concentrations rose following the infusion of 30 g of arginine hydrochloride in 15 of 33 acromegalic patients and during insulin-induced hypoglycemia in 12 of 20 patients. These responses, like those to glucose, were consistent in a given patient. For example, in patient N.P. the GH levels rose from 27 to 124 ng/ml and 50 to 118 ng/ml following arginine infusion on two occasions and in patient V.Y. the GH levels rose from 200 to 345 ng/ml, 41 to 224 ng/ml, 64 to 456 ng/ml and 60 to 123 ng/ml during insulin-induced hypoglycemia on four occasions.

Thus, changes in GH secretion following glucose ingestion, arginine infusion or insulin-induced hypoglycemia occurred in 31 of 50 acromegalic patients. Of the 14 patients in whom all three tests were performed, GH responses to at least one maneuver occurred in 12. These findings are comparable to those of Lawrence and coworkers (7) who found GH responses to hyperglycemia, hypoglycemia, arginine, or exercise in 20 of 24 acromegalic patients.

The GH secretory response to hypoglycemia is mediated through the hypothalamus. Hypothalamic lesioning (8) and intrahypothalamic glucose infusion (9) inhibit the GH response to hypoglycemia in monkeys. In man, pituitary

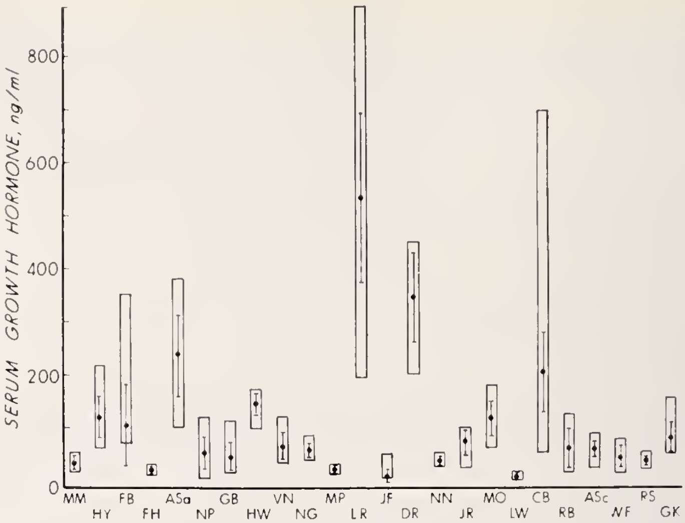

FIG. 1. Twenty-four hour serum GH values in 24 acromegalic patients. The points and brackets indicate the mean ± one standard deviation; the rectangles indicate the observed range.

stalk section blocks the GH response to hypoglycemia even when the capacity of the pituitary to secrete GH is preserved as evidenced by the presence of measurable circulating GH in the baseline state (10). It is often assumed that the GH secretory response to arginine is mediated through the hypothalamus but evidence comparable to that cited for glucose effects on GH secretion is lacking.

*Twenty-four-hour growth hormone profiles.* Hourly serum GH levels throughout a 24-hour period, drawn through an indwelling intravenous line, have been obtained in 24 acromegalic patients. As shown in Figure 1, mean serum GH concentrations over a 24-hour period ranged from 6 to 532 ng/ml. The median of the mean values was 60 ng/ml.

A striking feature of the 24-hour GH profiles was the marked variation in serum GH concentration in a given patient as noted previously (11). The observed values ranged up to 24-fold (5–119 ng/ml) during a 24-hour period in patient N.P. Of the 24 acromegalic patients studied, the coefficient of variation* of the serum GH concentration during a 24-hour period exceeded 25% in 18 patients and exceeded 50 percent in seven patients.

We have previously noted an apparent relationship between serum GH peaks and meals and physical activity during a 24-hour period in selected acromegalic patients (11). These patients exhibited post-prandial GH peaks and had higher serum GH levels during the day. However, a sharply defined serum GH peak following the onset of sleep, a characteristic feature of GH secretion in normal subjects (12), was not seen. This has also been the experience of others (13). However, we have recently studied a patient who had higher levels at night

* Standard deviation over the mean, expressed as a percentage.

than during the day on two occasions (Fig. 2). These rather broad nocturnal peaks may be analogous to the sleep-related spike in GH secretion observed in nonacromegalic normal subjects.

The composite GH responses of patient L.R. to oral glucose, intravenous arginine, insulin-induced hypoglycemia and the more physiologic stimuli to GH secretion exerted during a 24-hour sampling period are illustrated in Figure 3. Suppression of the serum GH level from 540 to 235 ng/ml followed glucose ingestion; the GH values rose from 240 to 725 ng/ml following arginine infusion and from 110 to 380 ng/ml during insulin-induced hypoglycemia. During the 24-hour profile the serum GH levels ranged from 80 to 500 ng/ml.

*Thyrotropin-releasing hormone (TRH) and L-dihydroxyphenylalanine (L-Dopa).* Synthetic TRH has been administered intravenously, and L-Dopa orally, to a small number of acromegalic patients. Examples are illustrated in Figure 4. GH secretory responses to TRH in acromegalic patients have been

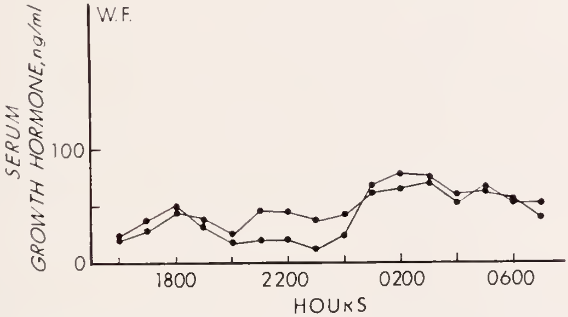

Fig. 2. Serum GH profiles obtained from acromegalic patient W.F. on two separate studies.

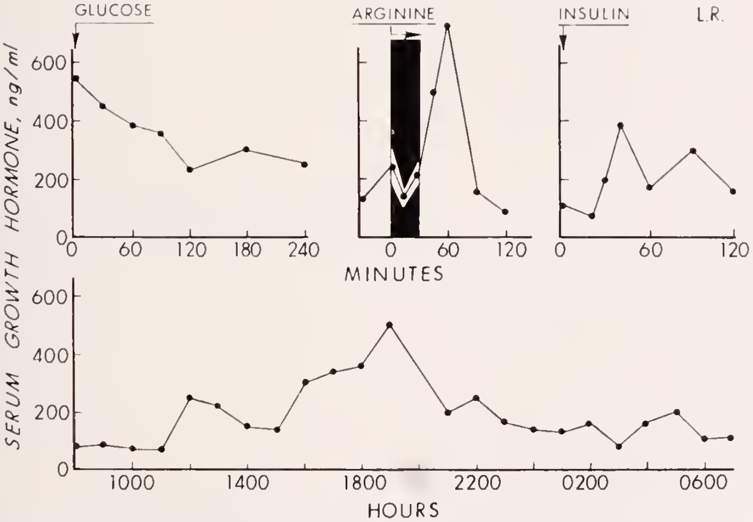

Fig. 3. Serum GH levels in acromegalic patient L.R. following oral glucose, intravenous arginine and insulin-induced hypoglycemia and during a 24-hour observation period.

reported by others (14); TRH does not stimulate GH secretion in normal subjects (15).

There is considerable evidence that alpha-adrenergic mechanisms trigger GH secretion (16–18). Studies in nonhuman primates suggest that this effect is exerted at the level of the hypothalamus rather than at the pituitary level (19). Whether adrenergic stimuli are major determinants or only modulating influences on GH secretion in normal subjects remains to be determined. However, the possibility that deranged adrenergic mechanisms may play a role in the pathologic physiology of acromegaly (20) is currently under investigation in our laboratory.

*Comment.* From these observations it is clear that the secretion of GH from the pituitaries of the majority of acromegalic patients is not autonomous. In most acromegalics qualitatively appropriate GH secretory responses occur following provocative manipulations, e.g., glucose ingestion, arginine infusion or insulin-induced hypoglycemia, and during the more subtle stimuli, e.g., meals, activity and others, exerted during a 24-hour observation period. Indeed, in one acromegalic patient, a sleep-related increase in GH secretion, the hallmark of normal GH secretion in nonacromegalic subjects, may have occurred. Thus, despite quantitatively excessive GH secretion, regulatory influences on GH secretion persist in most acromegalic patients.

In view of the intimate functional relationship between the hypothalamus

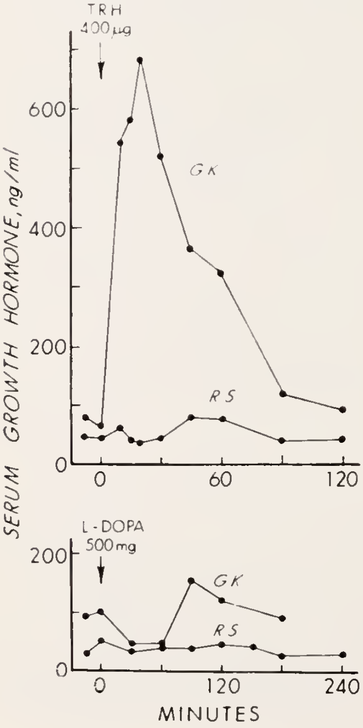

Fig. 4. Serum GH levels following intravenous thyrotropin releasing hormone and oral L-Dopa in acromegalic patients G.K. and R.S.

and pituitary GH secretion and the fact that the glucose-mediated (and perhaps other) GH secretory stimuli described above are mediated via the hypothalamus, we have hypothesized that a defect in hypothalamic regulation of GH secretion may underlie the development of acromegaly in some patients (11).

Diminished hypothalamic sensitivity to normal suppression of growth hormone releasing factor production is a potential mechanism for the putative regulatory defect in these acromegalic patients. Evidence that feedback suppression of GH secretion is operative in acromegalic patients has been adduced. The administration of estrogens, which block the effects of GH, has been shown to facilitate the GH secretory response to hypoglycemia in acromegalic patients (21). In addition, GH secretory responses to provocative stimuli in acromegalic patients with normal baseline serum GH levels after pituitary irradiation have been reported to be obliterated by the infusion of GH to produce serum GH levels comparable to those preceding therapy (22). Furthermore, Hagen, Lawrence and Kirsteins have detected bioassayable GH releasing activity in acromegalic plasma (23), an observation also reported by Strachma, Frohman and Dhariwal (24). Hagen and coworkers have observed increased GH-releasing activity in acromegalic plasma after hypophysectomy and decreased activity during effective medical therapy (25).

Thus, the concept of diminished hypothalamic sensitivity to (or elevated threshold for) normal suppression of GH releasing factor secretion, with resultant excessive production of releasing factor and GH, is consistent with available data. However, there are certain limitations to this simplified view. It is unlikely, for example, that fixed hypersecretion of GH releasing factor underlies the excessive GH secretion in these patients since, with serial GH determinations, some patients may, at times, have serum GH values in the normal range. Furthermore, it is unlikely that the defect is a failure of GH alone to feedback normally on hypothalamic GH releasing factor secretion since we have observed definite increments in serum GH in response to arginine in a patient whose baseline GH levels were normal following cryogenic hypophysectomy (11). Similarly, patients with normal serum GH levels following irradiation have been observed by others to respond to provocative secretory stimuli (22).

In many respects the excessive secretion of growth hormone in acromegaly resembles that encountered in Laron dwarfism (26). These individuals have high and variable concentrations of serum growth hormone (Fig. 5). They often respond to glucose administration and hypoglycemia with inappropriate changes in the serum GH level (Figs. 6 and 7). Growth hormone responses to arginine may be exaggerated. The hallmark of this condition is the low level of circulating somatomedin which fails to rise after growth hormone administration (27). The defects in this condition are best attributed to a generalized disorder of growth hormone receptors (26). Lacking the normal short-loop feedback inhibition of GH secretion the secretory pattern of an undamped system results in these patients. While pituitary tumors, as a result of pro-

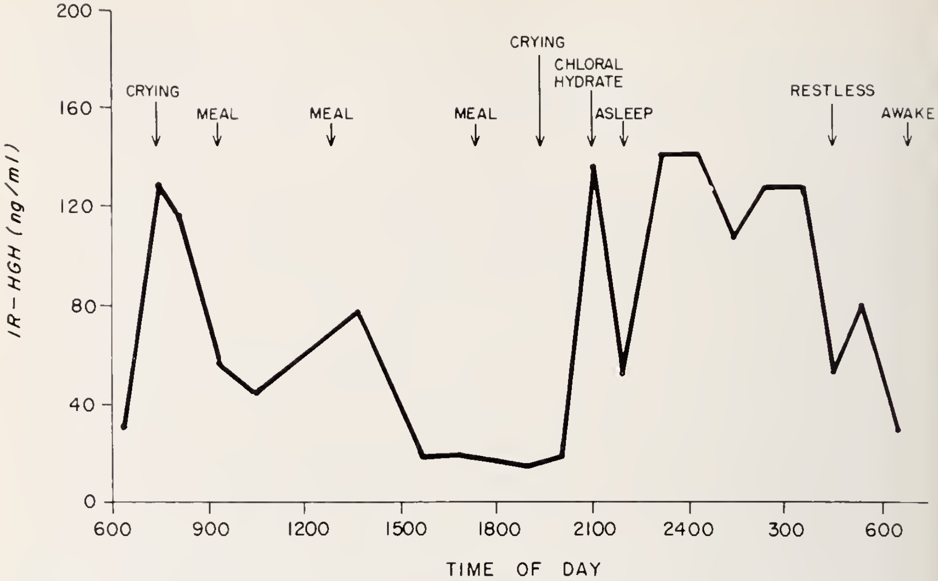

FIG. 5. Serum GH levels during a 24-hour observation period in a patient with Laron dwarfism (Reproduced with permission from Elders et al: J Pediatrics, In press.).

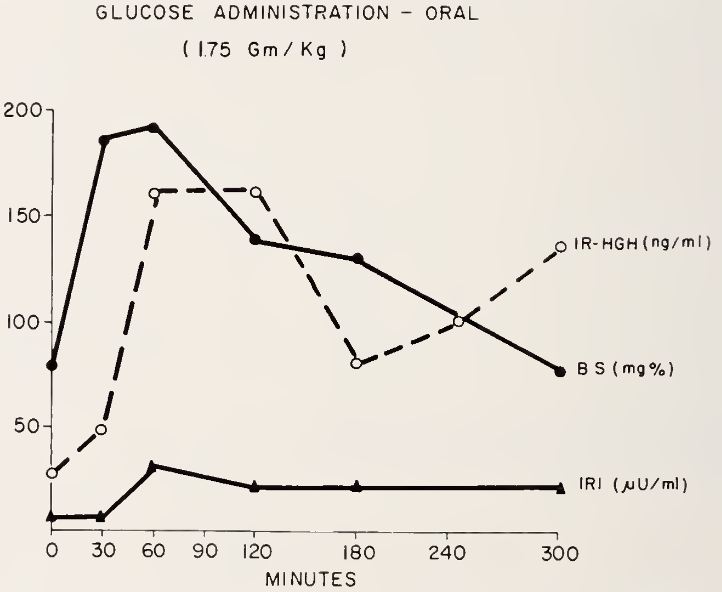

FIG. 6. Serum GH response to oral glucose in a patient with Laron dwarfism (Reproduced from Elders et al: J Pediatrics, In press.).

longed GH hypersecretion, are as yet unrecognized in Laron Dwarfs, the parallelism with growth hormone secretion in acromegaly is very suggestive. It is possible that the earliest disorder of GH secretion in acromegaly is a loss of short-loop feedback inhibition.

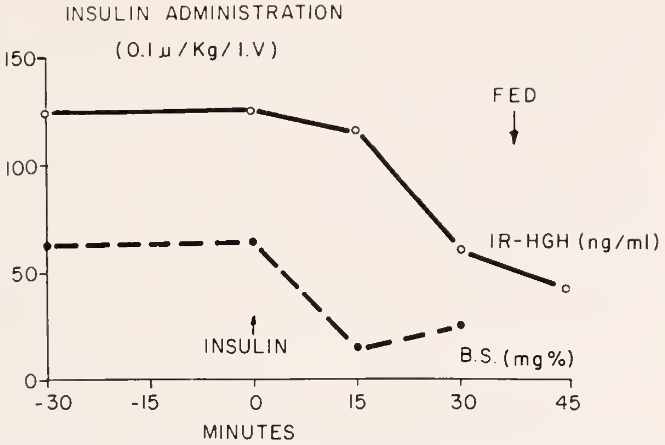

FIG. 7. Serum GH levels following insulin-induced hypoglycemia in a patient with Laron dwarfism (from Elders et al (26) with permission).

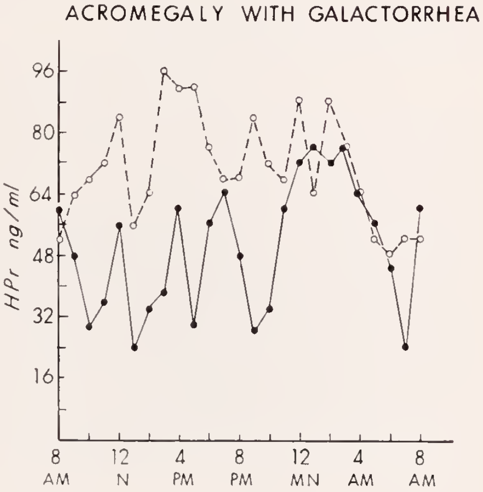

FIG. 8. Serum prolactin levels during a 24-hour observation period in two patients with acromegaly.

## *Regulation of Prolactin Secretion in Patients with Pituitary Tumors*

Excessive prolactin secretion is frequently encountered in patients with pituitary tumors (2, 28–32). While some patients with excessive prolactin secretion experience galactorrhea, often no manifestations are recognized clinically. Infrequently, excessive prolactin secretion is encountered in patients with acromegaly. In two such cases we have observed the same instability of prolactin secretion observed for growth hormone (Fig. 8).

We have now completed 24-hour profile studies in five patients with prolactin-secreting pituitary tumors. In four of these five a high degree of variance of the serum prolactin concentration was encountered (Fig. 9). In no case was the normal circadian nocturnal rise in serum prolactin recognized. It is of interest

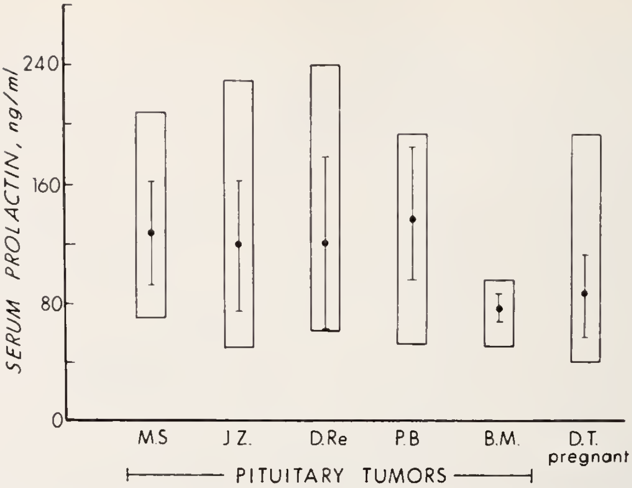

FIG. 9. Twenty-four hour serum prolactin levels in five patients with hyperprolactinemia and pituitary tumors and one normal subject with the physiologic hyperprolactinemia of late pregnancy. The points and brackets indicate the mean ± one standard deviation; the rectangles indicate the observed range.

that in the physiologic hyperprolactinemia of late pregnancy a similar variance of serum prolactin levels was observed (Fig. 9).

Prolactin secretion by pituitary tumors may be inhibited by the administration of L-DOPA (33, 34). We have given 0.5 g of L-DOPA orally to ten such patients (Fig. 10). Unequivocal suppression of serum prolactin levels followed L-DOPA in four patients, less definite falls were observed in three, and in three patients no significant change in serum prolactin occurred. In one patient, responsiveness to L-DOPA appeared after external irradiation to the pituitary. In three patients with prolactin secreting pituitary tumors no rise in serum prolactin followed the injection of 50 mg of chlorpromazine.

This experience with prolactin-secreting tumors appears to be analogous to that we have had with growth hormone-secreting tumors. A high degree of cyclic variation in secretion occurs and secretion is modified by agents acting through the hypothalamus.

## Summary

Reproducible changes in serum GH concentrations in the majority of acromegalic patients following oral glucose, intravenous arginine and insulin-induced hypoglycemia, and marked variation during the more subtle stimuli of a 24-hour observation period indicate that GH secretion, though quantitatively excessive, remains responsive to regulatory influences in these patients. Since at least some of these secretory stimuli are mediated through the hypothalamus, we have suggested that the primary defect in some acromegalic patients lies at the level of the hypothalamus rather than the pituitary. Reports of bioassayable GH-releasing activity in the plasma of acromegalic patients are consist-

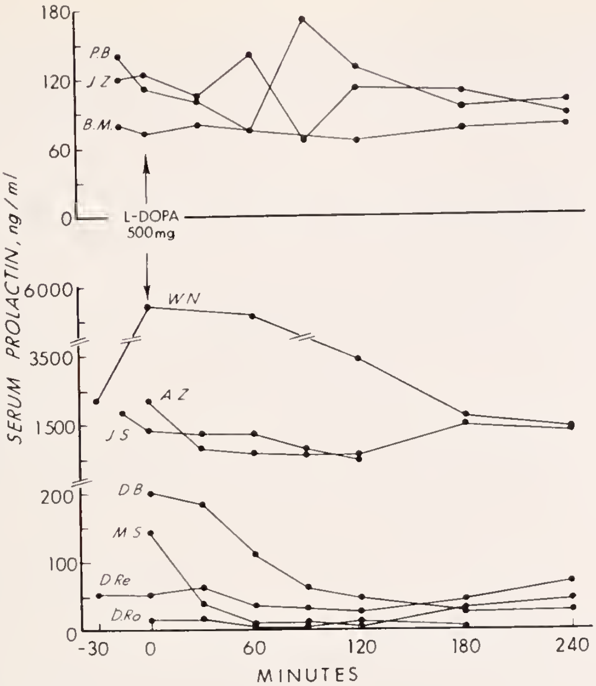

FIG. 10. Serum prolactin levels following oral L-Dopa in ten patients with hyperprolactinemia and pituitary tumors.

ent with the hypothesis that excessive GH releasing factor production from the hypothalamus underlies GH hypersecretion in these acromegalic patients.

Marked variation in the serum prolactin concentrations occurs during serial observation of patients with prolactin-secreting pituitary tumors. In some of these patients, the serum prolactin levels are suppressed following L-DOPA ingestion. Thus, there is considerable similarity between the patterns of GH and prolactin secretion in disorders characterized by pituitary tumors and excessive secretion of GH or of prolactin.

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# Evidence for Possible Precursors in the Synthesis of Growth Hormone by Rat and Human Fetal Anterior Pituitary in Vitro

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## Introduction

The existence of heterogeneity in the molecular size of polypeptide and protein hormones has been well established. Beginning with the studies of insulin by Steiner et al (1), gel filtration and immunologic techniques have demonstrated the presence of more than one form of several hormones both in the glands in which they are synthesized and in circulating plasma. The prototype of this model, for which there is the most conclusive evidence, is proinsulin (1, 2) though similar patterns have been demonstrated both with parathormone (3) and glucagon (4) biosynthesis. For each of these hormones, a pattern of heterogeneity has been observed in which a molecule somewhat larger than the native hormone serves as a hormone precursor, or prohormone, during the biosynthetic process.

It has also been established that the prohormone can be secreted. The large molecular-sized insulin ("big" insulin) observed in gel filtration fractionation of plasma (5) has now been accepted by most investigators to represent circulating proinsulin (6). Gel filtration has provided similar evidence for the heterogeneity of plasma parathormone (7), ACTH (8), and gastrin (9), although the patterns appear somewhat more complex.

The large-sized molecular forms of pituitary and placental hormones have been more variable. Extremely large molecular weight species have been described both for pituitary growth hormone (10, 11) and human placental lactogen (12), but their precursor role remains to be established. Although they constitute a relatively small fraction of total tissue hormone stores, these large molecular weight forms incorporate a considerable fraction of radioactivity during in vitro incubations in the presence of labeled amino acids. They have also been isolated from the incubation media in small quantities (11, 12).

Recently, heterogeneity in circulating GH has been reported from several laboratories (9, 13–15). Bala, Ferguson, and Beck (13) have reported that greater than 50 percent of plasma immunoreactive hGH appears in or near the void volume on Sephadex G-75 gel filtration while Berson and Yalow (9) observed a peak with a similar elution pattern (emerging prior to albumin) which comprised up to 30 percent of the total plasma hGH immunoreactivity. In contrast, Goodman, Tanenbaum, and Rabinowitz (14) and Gorden, Hendricks, and Roth (15) have found that approximately 20 to 30 percent of plasma growth hormone immunoreactivity is present in a form with a molecular

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size about twice that of the growth hormone monomer (i.e., 40,000 vs. 21,000 m.w.). Gel filtration of partially purified human pituitary GH preparations has also been shown to contain the larger molecular-sized GH forms (9, 14, 15) although at considerably lower concentrations.

In our previous studies on "large" growth hormone (11) there was the suggestion that some intermediate-sized forms did, indeed, exist. The experiments described in the present report provide evidence for the existence of intermediate-sized forms in the pituitary which appear to have a precursor role in growth hormone biosynthesis and which are of a size similar to that reported in plasma.

## Methods

### *Incubation Experiments*

*Rat.* Anterior pituitaries were obtained from 200 to 250 g male Holtzman rats, bisected, and incubated for varying time period in 1 ml Krebs-Ringer bicarbonate, pH 7.4, containing glucose 1.5 mg/ml and bovine serum albumin, 10 mg/ml and gassed with 95 percent $O_2$-5 percent $CO_2$. $^{14}$C-Leucine (S.A. 255 mCi/mM) or $^{3}$H-leucine (S.A. 32 Ci/mM) obtained from New England Nuclear was added in amounts described in the individual experiments. Upon completion of incubation, pituitary tissue was separated from the media, washed with cold buffer, homogenized in 0.01N NaOH, adjusted to pH 7.5 with 1N HCl, and centrifuged at 12,000 x g for 10 minutes.

*Human.* Human fetal pituitary, obtained from an early second trimester fetus, was similarly incubated in the presence of $^{3}$H-leucine for 4 hours and the tissue handled as above.

### *Gel Filtration and Growth Hormone Determination*

After adsorption with normal guinea pig serum, tissue extracts were filtered over Sephadex G-200 or G-75 columns 1 × 50 cm at 4°C and eluted with 0.05M phosphate buffer, pH 7.5. All columns were prewashed with 1 percent bovine serum albumin and the void volume determined with blue dextran. Flow rates were such that the void volume collection required from 7 to 10 hrs. Sample volume was always 1 ml or less.

Fractions of rat pituitary extract were reacted with excess specific monkey anti-rGH serum followed by goat anti-monkey gamma globulin serum, as previously described (11, 16) to determine the radioactive leucine incorporation into growth hormone. Normal monkey serum was substituted for immune monkey serum in control tubes. Fractions from the human pituitary experiment were similarly reacted using excess specific rabbit anti-hGH serum followed by goat anti-rabbit gamma globulin serum. Precipitates were washed once, dissolved in Soluene® (Packard) and counted in a liquid scintilation spectrometer at an efficiency of 45 to 50 percent ($^{3}$H) or 90 to 95 percent ($^{14}$C) using an external standard to correct for quenching. Radioimmunoassay of hGH was performed by a double antibody method as previously described (17).

## Results

### *Comparison of the Distribution Pattern of Rat Pituitary Immunoprecipitable Growth Hormone Radioactivity with Time*

Figure 1 illustrates the differences observed in the distribution of immunoprecipitable rGH radioactivity between anterior pituitaries incubated for 1 and 4 hours in the continuous presence of 5 μCi $^3$H- or .5 μCi $^{14}$C-leucine. The results from each time period represent the mean of four individual incubations and gel filtrations. The two previously reported (11) peaks of GH radioactivity appearing at the void volume ("large" GH) and at a Ve/Vo of 2.09 ("small" GH) were again noted. In addition, another peak was noted at a Ve/Vo of 1.61. The distribution of radioactivity in the three peaks varied between one and four hours with decreases noted in the large GH and intermediate GH peaks and an increase in the small GH peak.

The results of an experiment designed to trace the migration pattern of GH radioactivity with time is shown in Figure 2. Anterior pituitary glands were pulsed for five minutes with 50 μCi of $^3$H-leucine, transferred to fresh medium containing an excess of nonradioactive leucine, and incubated for an additional 155 minutes. Tissue removed at the end of the five-minute labeling period already showed the three previously described peaks, although improved resolution resulted in the demonstration of some heterogeneity in each peak. By the end of the chase period, some diminution in the large GH peak had occurred,

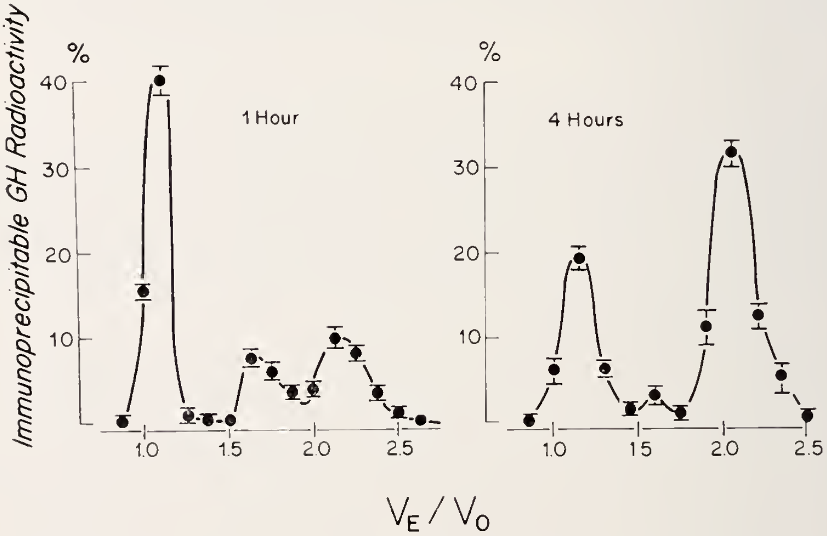

FIG. 1. Comparison of immunoprecipitable GH radioactivity distribution after one and four hours incubation of rat anterior pituitary in the presence of $^3$H- or $^{14}$C-leucine. Shown are the mean ± standard error of four separate incubations and gel filtrations over a single 1 × 50 cm Sephadex G-200 column. The ordinate represents the percent of total immunoprecipitable DPM recovered from the column. The volume of each fraction was 2 ml. The extracts consisted of approximately 6 mg anterior pituitary containing 9,000 to 30,000 DPM of $^3$H-GH or 4,000 to 15,000 DPM of $^{14}$C-GH.

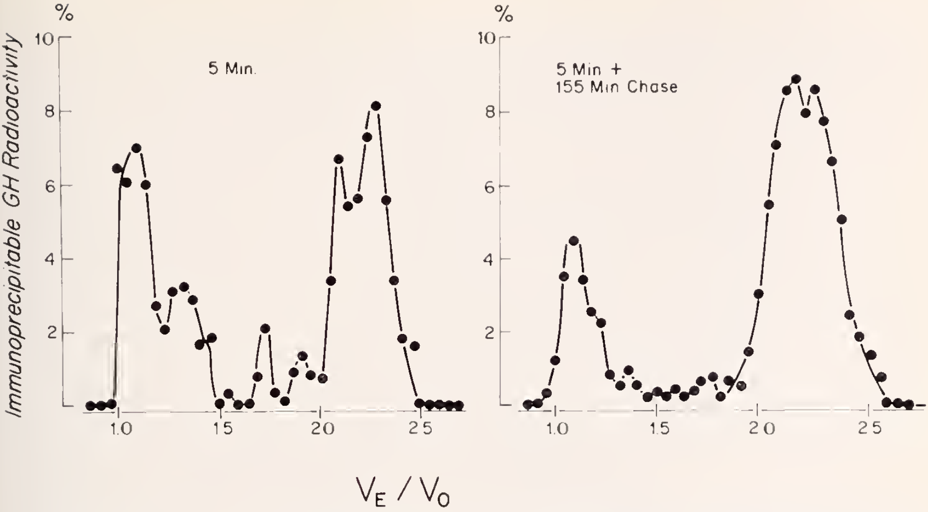

FIG. 2. Comparison of immunoprecipitable GH radioactivity distribution after five minutes incubation of rat anterior pituitary in the presence of $^3$H-leucine and after a subsequent 155 minute incubation in fresh media containing an excess of non-radioactive leucine. The extract of approximately 5 mg rat anterior pituitary containing 631 DPM/mg (five min) and 596 DPM/mg (160 min) $^3$H-GH was filtered over a single 1 × 50 cm Sephadex G-200 column. The ordinate represents the percent of total immunoprecipitable DPM recovered from the column. The volume of each fraction was 0.5 ml. Recovery of immunoprecipitable GH radioactivity was 93 and 89 percent, respectively. The $V_e/V_o$ of rabbit gamma globulin was 1.30 and that of bovine serum albumin was 1.49.

but, more significantly, the intermediate-sized GH peak had almost completely disappeared (7.5 to 2.7%). A corresponding increase in the small GH peak was again noted. The total radioactivity incorporated into immunoprecipitable GH was similar at five and 160 minutes (631 and 596 DPM/mg tissue respectively), indicating that the increase in the small GH radioactivity was absolute as well as relative. The size of the major component of the intermediate peak, based on the elution volumes of gamma globulin, albumin and small GH, was estimated to be approximately 40,000 m.w.

### *Distribution Pattern of Human Fetal Pituitary GH*

The distribution pattern of immunoprecipitable GH radioactivity and of total immunoreactive GH from a human fetal pituitary incubated for four hours in the presence of 3 $\mu$Ci $^3$H-leucine is shown in Figure 3. Three peaks were again noted, with the relative elution pattern being similar to that in rat pituitary tissue. An exact comparison with the results from the rat pituitary experiments was not possible because the human pituitary tissue extract was filtered over G-75 rather than G-200. The Ve/Vo of the intermediate (1.25) and small (1.60) GH peaks, however, were practically identical to the values obtained (1.30 and 1.62) from the filtration of clinical grade hGH (National Pituitary Agency, Lot No. 1-C) over G-75 (data not shown). Estimation of the

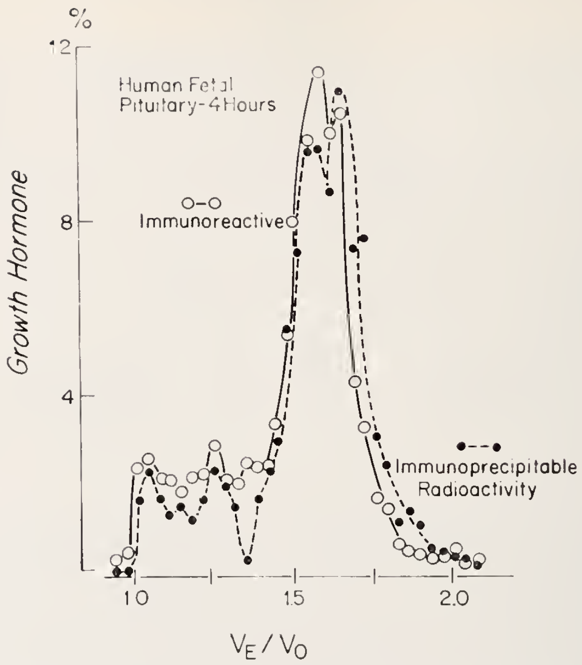

FIG. 3. Distribution of immunoreactive GH and immunoprecipitable GH radioactivity after four hrs incubation of human fetal pituitary in the presence of $^3$H-leucine. The extract of 0.35 mg anterior pituitary containing 12,400 DPM of $^3$H-GH was filtered over a 1 × 55 cm Sephadex G-75 column. The ordinate represents the percent of total immunoreactive GH or immunoprecipitable DPM recovered from the column. The volume of each fraction was 0.5 ml. The $V_e/V_o$ of bovine serum albumin was 1.06.

intermediate peak size based on the elution volume of albumin and of the small GH peak indicated a molecular weight of approximately 45,000.

The distribution of immunoreactive GH in the human fetal pituitary paralleled that of the $^3$H-GH, in contrast to the pattern previously reported for adult human pituitary where greater than 99 percent of immunoreactive GH was located in the small GH peak and no intermediate peak was apparent (11).

## Discussion

The experiments described provide evidence for the existence of multiple radioactive peaks with growth hormone immunoreactivity in rat and fetal human anterior pituitary incubated in the presence of radioactive leucine. Three major peaks, each with some heterogeneity, were noted: a large molecular weight peak, excluded from both Sephadex G-75 and G-200 ("large" GH); a smaller molecular weight peak ("small" GH) with an elution volume identical to that of the majority of the immunoreactive GH contained in the pituitary and to that of $^{125}$I-labeled GH (11); and an intermediate-sized peak with an elution volume slightly less than that of small GH.

The amount of radioactivity in both the intermediate and large GH peaks are shown to decrease with time in relation to that in the small GH peak during continuous incubation in the presence of radioactive leucine. The possibility that these GH forms may in some way be related to GH synthesis is suggested by the pulse-chase experiment in which the radioactivity of the intermediate GH peak noted after 5 minutes of incubation was reduced to almost zero and that of the large GH peak reduced by more than 50 percent by a 2.5 hr chase period.

The hypothesis that at least part of the radioactivity of large GH is contained in another protein with which GH is associated, is supported by the retention of considerable radioactivity in the large GH peak at the end of the chase period. This hypothesis has been suggested by other studies from this laboratory which demonstrated variations in the incorporation ratios of selected amino acids into large and small GH (18). Nevertheless, the decrease in radioactivity in the large and intermediate GH peaks was associated with an absolute increase in small GH radioactivity, indicating that this radioactivity was, indeed, associated with growth hormone itself.

The elution pattern of the intermediate hGH peak on G-75 is identical to that of the minor peak of GH immunoreactivity observed in partially purified hGH and is similar to that of "big" GH recently reported by Goodman et al (14) and Gorden et al (15) in both human plasma and partially purified hGH. These observations, therefore, raise the question of whether the intermediate peak is in fact identical to the plasma "big" GH and whether they represent a GH prohormone analagous to proinsulin. This question cannot be answered at the present. However, certain dissimilarities exist between the two systems. First, the distribution of GH radioactivity after a five-minute incubation pulse already revealed a small GH peak which contained nearly 50 percent of the radioactivity. A transfer of radioactivity through one or several intermediate steps would, therefore, have to occur very rapidly, and much faster than occurs in the conversion of proinsulin to insulin (1). Second, the spontaneous conversion of "big" GH to "little" GH upon freezing and thawing (14) is more suggestive of noncovalent binding than of a peptide bond such as exists between insulin and the connecting C-peptide. A noncovalent linkage of the GH molecule in the large molecular weight GH peak is also indicated by its partial dissociation in the presence of guanidine (11).

It is interesting to note that the relative percentage of the large and intermediate peaks of GH are increased in fetal as compared to adult human pituitary (11) and that fetal pituitaries of 12 to 20 weeks age contain only 2 to 4 μg GH/mg tissue (unpublished observations) which represents about ten percent of the adult concentration (19). This pattern, which is similar to that observed in the rat pituitary GH secreting tumor (11) which stores small quantities of GH when compared with normal pituitary tissue, suggests that the large and intermediate molecular weight GH peaks are involved in GH synthesis whereas the small GH peak represents stored hormone.

In summary, the data presented have confirmed the presence of an intermediary sized form of GH in rat and human pituitary gland similar to that which has recently been reported to exist in human plasma. By means of isotope incorporation experiments, it is suggested that the intermediary sized GH may represent a hormone precursor.

### Acknowledgments

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# Immunochemical Heterogeneity of Circulating Parathyroid Hormone in Man: Sequel to An Original Observation by Berson and Yalow

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It is not enough to say that I am pleased to have the opportunity to contribute to this volume of *The Mount Sinai Journal of Medicine* which has been dedicated to the memory of my teacher and friend, Sol Berson. As many others also must feel, my life has been permanently influenced by having known him. This paper is only a token of the tribute he deserves.

## Background

Berson and Yalow (1) were the first to apply their technique of radioimmunoassay to the measurement of parathyroid hormone (PTH) in human plasma. In their report, they described an assay which used an antiserum developed against bovine PTH, $^{131}$I-labeled bovine PTH as the labeled antigen, and an extract from a parathyroid adenoma as a standard. They showed that human PTH was immunologically different from bovine PTH ano were able to measure immunoreactive PTH in the plasma of two patients with primary hyperparathyroidism; the hormone was undetectable in the plasma of nine patients with clinical hypoparathyroidism.

Three years later, they followed up this work with the first systematic study (2) of plasma immunoreactive PTH in adenomatous hyperparathyroidism, the secondary hyperparathyroidism of chronic renal failure, and carcinoma of the lung without hypercalcemia. With an assay which was more sensitive than the one originally described and using hyperparathyroid plasma as a standard, they were able to measure immunoreactive PTH in the plasma of 60 percent of normal subjects. They showed that induced hypocalcemia and hypercalcemia caused increases and decreases, respectively, in plasma immunoreactive PTH in normal volunteers. Plasma immunoreactive PTH was increased above normal in 60 percent of patients with adenomatous hyperparathyroidism, in approximately 40 percent of normocalcemic patients with bronchogenic carcinoma, and in almost all patients with chronic renal failure and blood urea nitrogen concentrations above 50 mg/100 ml. In the patients with renal failure, they observed that the increase in plasma immunoreactive PTH appeared to be more closely related to the severity of the uremia than to the degree of hypocalcemia.

In 1968, Berson and Yalow published an extremely important paper (3) which presented strong evidence in support of the immunochemical heterogene-

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ity of the PTH in human plasma. They showed that one of their anti-bovine-PTH sera distinguished between the hormone extracted from parathyroid adenomata and that present in the peripheral serum of patients with primary hyperparathyroidism and that values for plasma immunoreactive PTH differed markedly depending on the antiserum used in the radioimmunoassay. Their suggestion that these phenomena reflected immunoheterogeneity of plasma PTH was based primarily on the observation that the measured half-life of disappearance of plasma immunoreactive PTH after parathyroidectomy in a patient with secondary hyperparathyroidism and chronic renal failure was different with two different antisera. They interpreted their data as indicating the presence of metabolites of PTH rather than of isohormones of PTH in the peripheral circulation of hyperparathyroid man.

At about the time this paper appeared, Reiss and Canterbury (4) reported the development of a radioimmunoassay for PTH in human serum which was capable of measuring the immunoreactive hormone in all normal subjects and of distinguishing clearly between normals and patients with adenomatous hyperparathyroidism. This latter observation was in sharp contrast with that of Berson and Yalow (2) who found a large overlap in plasma immunoreactive PTH values between these two groups. It is likely that this apparent discrepancy was the first clue that the phenomenon of immunochemical heterogeneity of plasma PTH has great practical significance in the application of the radioimmunoassay of PTH to problems of clinical diagnosis and investigation.

Arnaud and coworkers (5, 6) confirmed the observations of Berson and Yalow regarding the immunoheterogeneity of serum PTH by showing that antisera developed against both bovine and porcine PTH were able to distinguish between the PTH in extracts of parathyroid adenomata and that in hyperparathyroid serum. In addition, they (7) showed that the PTH present in crude urea extracts of parathyroid adenomata was immunoheterogeneous. Although these authors suggested that the immunochemical differences they observed might be due to artifacts of extraction, it is clear now that they probably reflected the presence of precursor molecules of PTH (8) in the crude extracts.

The second clue that immunochemical heterogeneity of PTH in the peripheral circulation had practical importance came from another discrepancy between results obtained with different antisera in different laboratories. Reiss and Canterbury (9) reported that it was possible to distinguish between adenoma and the primary hyperparathyroidism due to parathyroid hyperplasia by measuring serum immunoreactive PTH during induced hypercalcemia. With this manipulation, hormone concentrations decreased in patients with hyperplasia but did not in patients with adenoma. In contrast, using their own radioimmunoassay, Potts and coworkers (10) found that serum immunoreactive PTH increased with induced hypocalcemia and decreased with induced hypercalcemia in patients with adenomatous hyperparathyroidism.

I do not believe that this second discrepancy, or the first relating to the overlap of serum immunoreactive PTH values in normal and hyperparathyroid subjects, was due to differences in experimental procedure used in the different laboratories or to the use of reagents derived from a nonhuman species (bovine in each instance). As will be shown below, it is likely that the key variables in the explanation are antibody specificity and differences in the rates of metabolism of the various molecular species of PTH in the circulation. This notion is implicit in the paper by Berson and Yalow (3) describing plasma PTH immunochemical heterogeneity.

Canterbury and Reiss (11, 12) have recently shown, by gel filtration of Amicon-filtered, pooled, peripheral hyperparathyroid serum, that there are at least three immunoreactive forms of PTH in this serum. One form was eluted from Bio-Rad P-10 columns along with $^{131}$I-labeled bovine PTH (84 amino acids; mol wt, 9,500) and had a half-life of less than 30 minutes, whereas the other two forms (approximate mol wt, 7,000 to 8,000 and 4,000 to 5,000) had half-lives greater than 30 minutes. Habener and coworkers (13) confirmed these observations on peripheral hyperparathyroid serum except that the antiserum used in their radioimmunoassay apparently did not detect the 4,000 to 5,000 mol wt form. These latter workers (13) also examined the plasma from the venous drainage of the thyroid gland in hyperparathyroid patients. They found primarily the 9,500 mol wt form of the hormone in this plasma, indicating that this is a major from of the hormone secreted by parathyroid adenomata and that the smaller forms of PTH present in general circulation are probably derived from metabolic degradation of it.

Most recently, Segre and coworkers (14) studied hyperparathyroid serum by using radioimmunoassays based on anti-bovine-PTH sera preadsorbed with fragments of bovine PTH and presumably specific for the amino terminal (residues 14 through 19) and carboxy terminal (residues 53 through 84) regions of the bovine PTH molecule. They found that most of the immunoreactivity in these sera was associated with the carboxy terminal region. Because the synthetic amino terminal fragment (residues 1 through 34) of bovine PTH is biologically active but the carboxy terminal fragment (residues 53 through 84) obtained from natural sources is biologically inert (15), these workers concluded that the major portion of the circulating PTH is biologically inactive. However, in this work also, the antisera did not detect the 4,000 to 5,000 mol wt circulating immunoreactive PTH fragment previously reported by Canterbury and Reiss. In a current paper, Canterbury et al (16) report that this fragment obtained from hyperparathyroid serum has biologic activity in the rat renal adenyl cyclase assay system.

## Recent Results

We have taken a pragmatic approach to evaluation of the radioimmunoassays of human serum PTH. Our purpose has been to determine the practical importance of the immunoheterogeneity problem in the application of the PTH radioimmunoassay to clinical medicine. Radioimmunoassays with two antisera of widely different specificities have been used in two basic types of study. In one type, immunoreactive PTH in serum or plasma was measured with both assays during the steady state in large numbers of patients with primary hyperparathyroidism due either to adenoma or hyperplasia or with secondary hyperparathyroidism due to renal failure. Osteoclast numbers were also quantitated in bone biopsy specimens obtained from some of the patients with primary hyperparathyroidism. In the second type, immunoreactive PTH in plasma was measured with both assays during induced hypercalcemia in patients with end-stage chronic renal failure who were being treated with chronic hemodialysis at the time. The details of this work are available elsewhere (17, 18), and the emphasis here will be on the results of these studies.

### *Steady-State Immunoreactive PTH in Hyperparathyroidism*

The antisera used in these studies were characterized by determining their relative affinities for human PTH purified from urea extracts of parathyroid adenomata and for the total PTH complement present in sera obtained from a patient with severe primary hyperparathyroidism; they also were examined for their ability to recognize the various immunoreactive species of PTH contained in fractions of gel-filtered (Bio-Rad P-30) hyperparathyroid serum. Both antisera had identical affinities for adenoma-extracted PTH (presumably human PTH, residues 1 through 84). However, antiserum GP 1M, which was developed against porcine PTH in a guinea pig, recognized the total PTH complement in hyperparathyroid serum ten times better than did the other antiserum, CH 14M, which was developed against bovine PTH in a cockerel. The reason for this became apparent in the investigations of hyperparathyroid serum by means of gel filtration.

Antiserum GP 1M recognized not only the 9,500 mol wt form of the hormone but also a much larger immunoreactive fraction whose components ranged from 3,000 to 8,000 mol wt, whereas antiserum CH 14M recognized only the 9,500 mol wt form and a small component (2,000 to 4,000 mol wt). The explanation for these discordant results resided, therefore, in the presence of a large immunoreactive fraction in serum with a molecular weight less than 9,500 and with which one antiserum reacted (GP 1M) and the other (CH 14M), for practical purposes, did not.

When radioimmunoassays using these two antisera were applied to the study of sera from normal subjects and patients with hyperparathyroidism, there were striking differences in the results obtained with each antiserum. In patients with end-stage chronic renal failure, assays with GP 1M (recognizing components 3,000 to 9,500 in mol wt) gave serum immunoreactive PTH values ranging from 3 to 200 times normal in all patients. However, assays with CH 14M (not recognizing the 4,000 to 8,000 mol wt components) showed increases in only 70 percent of the same patients and these increases ranged from barely above the upper limit of normal to four times normal. In patients with primary hyperparathyroidism, a similar phenomenon was observed: serum immunoreactive PTH was increased above normal in 90 percent of patients when antiserum GP 1M was used but in less than 70 percent of the same patients when antiserum CH 14M was used. These observations are similar to the various degrees of overlap in serum immunoreactive PTH values between normal and primary hyperparathyroidism observed by others using different radioimmunoassays. Our results suggest that the phenomenon of overlap is primarily related to the presence, in hyperparathyroid serum, of a large amount of immunoreactive PTH components ranging from 4,000 to 8,000 in mol wt and the different abilities of various antisera to recognize this fraction.

Perhaps the most important aspect of these studies comparing assays of serum by these two antisera are the correlations we obtained between serum immunoreactive PTH and osteoclast counts on thin sections of bone obtained from many of the patients with primary hyperparathyroidism. The osteoclast counts increased uniformly with increases in serum immunoreactive PTH when assays were done with antiserum GP 1M whereas this relationship was only gross when assays were done with antiserum CH 14M. In fact, significant increases in osteoclast count were observed in 4 patients (of a total of 18) who had a normal serum immunoreactive PTH value as measured in assays using antiserum CH 14M whereas all patients with increased osteoclast counts had an increased serum immunoreactive PTH value as measured in assays using antiserum GP 1M. These results suggest that, in hyperparathyroid serum, measurement of the large immunoreactive PTH fraction with mol wt 4,000 to 8,000 is a better reflection of the chronic target tissue response to PTH than is measurement of the 9,500 mol wt component.

### *Plasma Immunoreactive PTH during Induced Hypercalcemia in Hemodialyzed Patients with Chronic Renal Failure*

We studied 16 patients who were undergoing chronic hemodialysis for end-stage renal failure; four- or eight-hour calcium infusions were performed and serum immunoreactive PTH was measured with both antiserum GP 1M and antiserum CH 14M (Fig. 1). In support of the data described in the previous section, control values for serum immunoreactive PTH were uniformly higher than normal with antiserum GP 1M but were normal in 12 of 19 studies with antiserum CH 14M. Suppressive responses to induced hypercalcemia (mean maximal serum calcium, 14 mg/100 ml) with assays using antiserum GP 1M were variable and the mean changes from control were not significant. In contrast, serum immunoreactive PTH decreased significantly during every calcium infusion when assays with antiserum CH 14M were used (mean decrease, 50%). Because antiserum CH 14M recognizes primarily the 9,500 mol wt form of circulating immunoreactive PTH and because this form appears to have a short half-life in serum (12) and be a major secretory product of the parathyroid gland, we have concluded that assays of serum immunoreactive PTH using antiserum CH 14M reflect primarily the moment-to-moment secretory status of the parathyroid glands. It is likely that the discrepancy between the results described by Reiss and Canterbury and those of Potts and coworkers regarding the "autonomy" of parathyroid adenomata can be explained on the basis of different antisera specificities and are analogous to the observations just described.

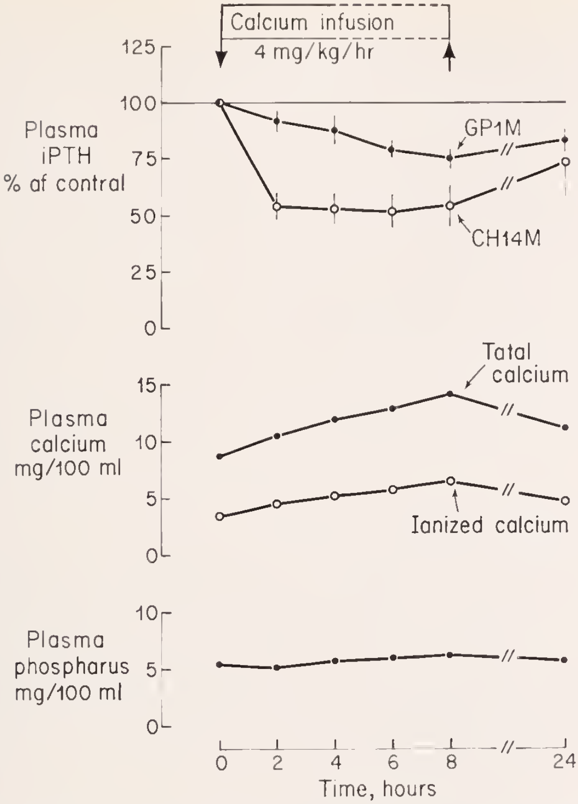

FIG. 1. Plasma total and ionized calcium, phosphorus, and immunoreactive parathyroid hormone, as means ± SE, before, during, and after 8-hour calcium infusion in 14 patients with end-stage renal failure being treated with chronic hemodialysis. Values for plasma immunoreactive PTH measured with antiserum GP 1M (3,000 to 9,500 mol wt species) and antiserum CH 14M (primarily 9,500 mol wt species) are expressed as percentages of control values. For total calcium, ionized calcium, and phosphorus, SE was less than the height of the symbols showing means. (From Goldsmith, R. S., Furszyfer, J., Johnson, W. J., Fournier, A. E., Sizemore, G. W., and Arnaud, C. D.: Etiology of Hyperparathyroidism and Bone Disease during Chronic Hemodialysis. III. Evaluation of Parathyroid Suppressibility, J Clin Invest 52:173, 1973. By permission of the American Society for Clinical Investigation.)

### *Human Parathyroid Hormone: Amino Acid Sequence of Residues 1 through 34*

Only nonhuman species of parathyroid hormone (bovine and porcine) have been available for the production of antisera and for use as $^{125}$I- and $^{131}$I-labeled species in radioimmunoassays of PTH in human serum. Structural differences among the three species have been predicted from immunologic investigations (19) and these structural differences undoubtedly have had an adverse influence on the development of assays of human hormone, based on the cross-reaction between human PTH and bovine and porcine PTH, sufficiently sensitive for the measurement of the low concentrations of PTH in serum. Additionally, workers have been limited to the use of bovine PTH fragments (14) or the PTH in purified preparations of parathyroid adenoma extracts (probably the amino acid sequence 1 through 84) and ill-defined presumed fragments of this molecule, obtained from gel filtration of hyperparathyroid serum, to characterize the antigenic determinants of antisera.

In a recent collaborative effort between our laboratory and the laboratory of Dr. Brian Brewer (National Institutes of Health), the sequence of the amino terminal residues (amino acids 1 through 34) of human parathyroid hormone was determined (20). A comparison of the primary structures of this region of the human, bovine, and porcine molecules is shown in Figure 2 (the structures of bovine (21, 22) and porcine (15) PTH have been taken from the literature). The amino acid sequence of the amino terminal region of human PTH differs from bovine PTH in six places and from porcine PTH in five places. However, the differences from the bovine molecule are distributed throughout the length of the segment whereas the differences from the porcine molecule are concentrated after residue 15. Thus, residues 1 through 15 are identical in the human and porcine molecules, even to the amino terminal residue, serine; in bovine PTH, the amino terminal residue is alanine.

Based on the amino acid sequence shown in Figure 2, the amino terminal 34 residue sequence of human PTH has been synthesized (23). Preliminary work has shown that this synthetic peptide causes hypercalcemia and phosphaturia in the conscious, thyroparathyroidectomized, perfused rat and stimulates rat renal adenyl cyclase in vitro. We have recently labeled it with $^{131}I$ and have found that it binds to antibody when incubated with either antiserum GP 1M or antiserum CH 14M.

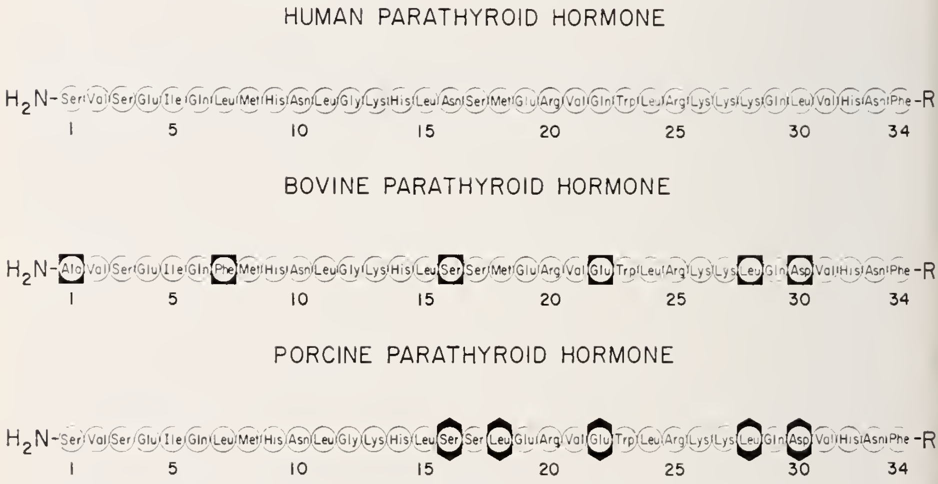
HUMAN PARATHYROID HORMONE
H2N-Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Asn Ser Met Glu Arg Val Gln Trp Leu Arg Lys Lys Lys Gln Leu Val His Asn Phe -R
1 5 10 15 20 25 30 34
BOVINE PARATHYROID HORMONE
H2N-Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu Ser Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe -R
1 5 10 15 20 25 30 34
PORCINE PARATHYROID HORMONE
H2N-Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu Ser Ser Leu Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe -R
1 5 10 15 20 25 30 34

Fig. 2. Comparison of the amino acid sequences of amino terminal 34 residues of human, bovine, and porcine parathyroid hormones. (From Brewer, H. B., Jr., Fairwell, T., Ronan, R., Sizemore, G. W., and Arnaud, C. D.: Human Parathyroid Hormone: Amino-Acid Sequence of the Amino-Terminal Residues 1–34, Proc Natl Acad Sci USA 69:3585, 1972. By permission of the National Academy of Sciences.)

## Discussion

The evidence currently available clearly establishes that there are at least three circulating forms of immunoreactive PTH in the serum of patients with hyperparathyroidism. One, the 9,500 mol wt form that presumably represents the first 84 amino acids of parathyroid hormone, is a major secretory product of abnormal parathyroid tissue but appears to comprise a relatively small proportion of the total complement of PTH immunoreactivity in hyperparathyroid subjects. We have described an antiserum to bovine PTH (CH 14M) which recognizes this form as well as a species with mol wt between 2,000 and 4,000. Because of the relatively small concentrations of these forms in serum, assays with antisera of this type must have great sensitivity to be useful in the measurement of immunoreactive PTH in serum. (Recent studies in our laboratory indicate that the assay which uses antiserum CH 14M and which measures immunoreactive PTH in greater than 95% of normal sera detects as little as 20 to 50 pg of highly purified human PTH.)

The high degree of specificity intrinsic to this type of assay and the apparent rapidity with which the immunoreactive form of PTH it measures disappears from the circulation make it ideal for acute studies of parathyroid secretion and possibly for localization of parathyroid lesions by assaying serum specimens obtained during differential catheterization of the venous drainage of the neck and mediastinum. However, the results of our comparative studies of patients with hyperparathyroidism indicate that an assay which uses an antiserum that is highly specific for the 9,500 mol wt species of circulating PTH is far less useful for assessing the chronic steady-state status of parathyroid function than is an assay which uses an antiserum that is less restricted in its ability to react with circulating species of PTH. A precise immunochemical explanation of this phenomenon is not at hand at the present time except that assays using antisera of the latter type come close to measuring all circulating forms of PTH—forms which differ widely in their rates of metabolism and disposal—whereas assays using antisera of the former type measure primarily one form which exists in serum in relatively low concentration and has a rapid turnover. The practical relevance of these observations in the development of radioimmunoassays of PTH to be used for assessing steady-state serum concentrations of immunoreactive PTH is that antisera should be selected on the basis of their ability to react with serum PTH, not PTH extracted from adenomata.

A definitive approach to determining the specificity of various antisera for the biologically active region of the human PTH molecule (the amino terminal 34 amino acids) now is possible. In fact, sufficient quantities of synthetic human PTH (amino acids 1 through 34) should be available soon for the development of species-specific radioimmunoassays of human PTH. This assumes that the amino terminal region of human PTH is sufficiently antigenic to induce antibodies in animals and that a good quality $^{131}$I- or $^{125}$I-labeled synthetic human PTH 1-34 preparation can be produced. In this regard, the absence of a tyrosine residue in this sequence does not appear to be a problem because it is likely that the histidine residues iodinate well. However, a synthetic peptide containing a carboxy terminal tyrosyl residue would be a welcome reagent.

A note of caution should be introduced in the current enthusiasm regarding the potential for developing species-specific assays for human PTH by using synthetic human PTH 1-34. It may be that the work presented in this paper on the comparative usefulness of two assays using antisera of widely different specificities may portend difficulties. It is entirely possible that immunologic recognition of the carboxy terminal region of the PTH molecule in serum may be essential in the development of assays similar to the one described with antiserum GP 1M which appears to be superior to others in assessing chronic steady-state parathyroid function. If so, ultimately it may be necessary to have synthetic preparations of the carboxy terminal region of the human PTH molecule available for immunization purposes.

This paper has not considered the possibility that precursors of the PTH molecule may be major secretory products of parathyroid tissue. Evidence that these exist in the parathyroid cell is abundant (8, 24–26) but progress must be made in the study of the chemistry of these molecules before specific studies of their secretion in vivo can be attempted. It is a good bet, however, that a component of plasma PTH immunoreactivity will be found that is composed of a precursor molecule(s).

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# Berson and Yalow's Radioimmunoassay for Parathyroid Hormone (PTH): A Clinical Progress Report

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The scientific contributions of Sol Berson have already achieved the status of classics. Surely radioimmunoassay, which he and Rosalyn Yalow originated, pioneered, perfected, and applied so well to elucidate clinical and fundamental problems, must always be recorded as one of the great advances in science. The future reader may find it more difficult to detect from recorded writings Sol's equally great stature as a *Mensch* of high standards, unimpeachable integrity, human consideration and compassion, deep-felt friendships, and a paragon of scholarship and culture. A superb clinician and teacher, he was also a veritable Renaissance *uomo universale*. We were fortunate enough to know and to collaborate with Sol and, like all who came within his circle, we were influenced more than pen can convey by his warmth, insight, knowledge, and brilliance.

In endocrinology, the radioimunoassay of Berson and Yalow provides direct, sensitive, highly specific measurement of peptide and other hormones which formerly were either poorly measureable or not at all. Of the various radioimmunoassays, that for parathyroid hormone has turned out one of the most difficult and has been mastered in but a few laboratories. With the considerable help of Sol Berson and Rosalyn Yalow, we have had the good fortune to establish this radioimmunoassay in our laboratory, to validate it in primary and secondary hyper- and hypoparathyroidism and to use it as a tool to investigate the pathologic physiology of various hypo- and hypercalcemias, bone diseases, and malignant tumors.

Before considering the empiric results obtained in various clinical conditions, it is essential to consider a most important general principle which now appears to hold for all peptide hormones (1). In 1968, Berson and Yalow noted that their antisera identified in plasma two different immunoreactive parathyroid peptides, one of which disappeared rapidly after removal of a parathyroid adenoma with a half-life of 12 to 20 minutes, and another with a longer half-life of one and one-half to two hours (2). This heterogeneity in plasma was subsequently found to reflect different parathyroid peptides which are separable by physicochemical techniques that differentiate molecules of different size. It has been proposed that the larger molecule represents the form of the hormone in the gland, covalently bound to an additional peptide, and the smaller a circulating hormone derived from the larger by cleavage of the additional peptide. On the other hand, it is worth remembering that Berson and Yalow first demonstrated both of these molecular species in plasma. Evidence of the

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nature of these molecules, though still in a formative stage, suggests an analogy to insulin which is elaborated as proinsulin, from which the connecting peptide is cleaved to yield the smaller molecule. Normally, the larger primordial proinsulin molecule is almost all cleaved before reaching the blood so that it comprises no more than 15 to 20 percent of the circulating insulin. In some, but not all, insulinomas, on the other hand, most or all of the circulating hormone is in the larger pro-insulin form. Further ideas about the nature of the glandular and secreted parathyroid hormones are expressed elsewhere in this issue. Immunochemical heterogeneity has been established by Berson and Yalow for glucagon, gastrin, growth hormone and ACTH as well as PTH and insulin (1). Regardless of the exact nature of the heterogeneity, it is immediately apparent that the amount of peptide hormone recognized by any given antibody is necessarily a function of its ability to recognize the various circulating molecular species, as well as the amount of each species present. Thus, absolute numbers previously derived from radioimmunoassay, including those in this presentation, are necessarily incomplete. Fortunately, the guinea pig antibody to PTH we routinely use seems to recognize both of Berson and Yalow's molecular species equally well.

Using partially purified beef PTH and complete Freund's adjuvant, we have immunized 74 guinea pigs and 35 chickens. The antibodies in both species recognize the usual reference materials (Fig. 1). These are highly purified bovine PTH, the human hormone extracted from parathyroid adenomas, the human material circulating in patients with severe renal insufficiency, and the hormone in the serum of patients with primary hyperparathyroidism. The ratio of bound to free tracer (B/F) is reduced by 30 percent when 0.5 ng of bovine PTH 1000 USP u per mg (Wilson & Co.) is used, and by 84 percent when 0.5 ng of bovine PTH 3000 USP u per mg prepared in our laboratory is used. Lacking highly purified human PTH as a routine reference standard, we deem it misleading to report results in nanograms of bovine material since the immunoreactivity varies in different conditions. We, therefore, report results as $\mu$l equivalents of a standard extract of human parathyroid adenoma, 20 $\mu$l of which has the same immunoreactivity as 1.0 ng of Wilson's highly purified bovine PTH or 0.8 ng of a highly purified human PTH preparation kindly supplied by Dr. Claude Arnaud.

It should also be recalled that parathyroid secretion changes rapidly, being inhibited by slightly increased concentrations of calcium and stimulated by hypocalcemia.

We find higher levels in children than in adults (Table I), and this difference is already evident at birth. In 340 children, from birth to age 17, even when subdivided by sex and year of age, all mean values were higher than those in adults. With advanced age there is a mild stepwise decline with each decade past age 50. Arnaud has described a circadian rhythm, with a slight rise in the serum level during the day (3). We also find that levels of uremic patients are higher after noon than in the morning but the changes are not of sufficient magnitude to interfere with ordinary clinical diagnostic purposes.

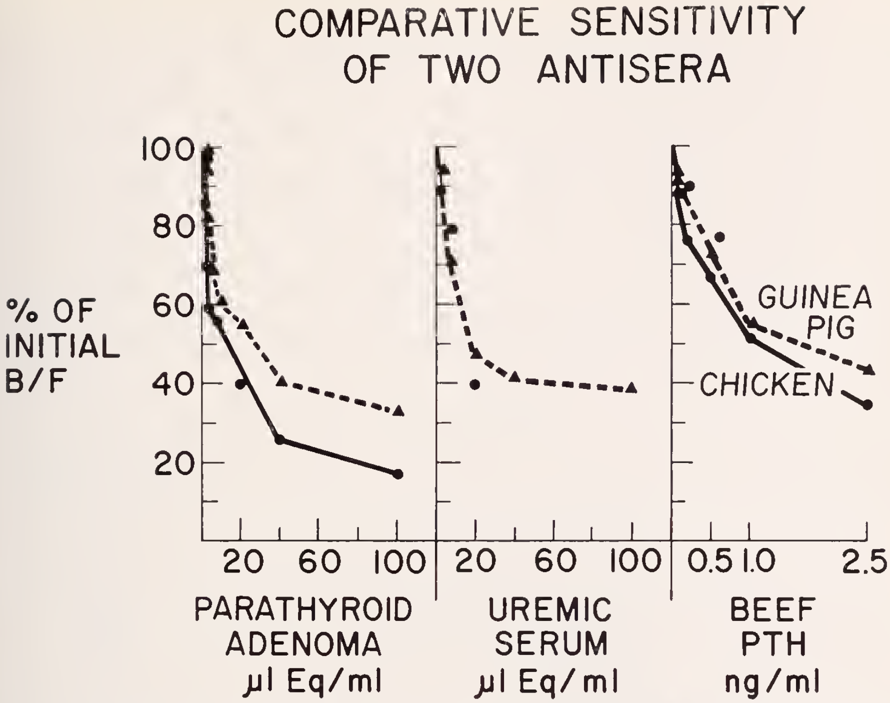

FIG. 1. Comparison of sensitivities of a guinea pig antiserum (▲) and a chicken antiserum (●) both evoked with beef PTH. Initial B/F of guinea pig antiserum 1.0, chicken antiserum 0.8. Left: Extract of an adenoma weighing 17 gm. prepared by homogenizing and extracting with acetone-acetic acid, centrifuged and the supernatant diluted 1:50. Center: Uremic serum from young woman with chronic renal disease and severe osteodystrophy. Right: Beef PTH (Wilson and Co.) 1000 USP u/mg.

Serum immunoreactive PTH levels are, as one might expect, low or nil in surgical or idiopathic hypoparathyroidism (Table II). It should be emphasized that zero values are also found in 15 percent of normal adults and in 25 percent of normal children. The larger number of zero values in children probably relates to their milk intake. Since idiopathic hypoparathyroidism may be an autoimmune disease, we had hoped that the serum of these patients might serve as a spontaneous antiserum to human PTH. Unfortunately, using immunoelectrophoresis and the Ouchterloney technique, we have not been able to detect antibodies to PTH in the serum of patients with idiopathic hypoparathyroidism, although Blizzard, using immunofluorescence, has found antibodies to human parathyroid gland tissue (4).

The radioimmunoassay has been of enormous value in the differential diagnosis of several conditions other than hypoparathyroidism which are also associated with hypocalcemia and hyperphosphatemia. In contrast to idiopathic hypoparathyroidism where values are at or near zero, the levels of patients with pseudohypoparathyroidism are regularly elevated because of the hypocalcemia. High levels correlate well with pathognomonic evidence of hyperparathyroid bone disease found by x-ray in some of these patients by Kolb and Steinbach

TABLE I

*Normal Serum PTH Values*

| No. | Group | PTH µl eq/ml ± S.E. |
|---|---|---|
| 389 | Children, age 0–17 | 25 ± 2 |
| 60 | Adults, age 20–49 | 20 ± 2 |
| 50 | age 50–59 | 9 ± 1 |
| 100 | age 60–69 | 4 ± 1 |
| 68 | age 70–79 | 2 ± 1 |
| 5 | age 80+ | 3 ± 1 |

TABLE II

*Hypoparathyroidism*

| No. | Group | PTH µl eq/ml ± S.E. |
|---|---|---|
| 14 | Postsurgical hypoparathyroidism | 5 ± 1 |
| 3 | Postsurgical with uremia | 0 |
| 10 | Idiopathic hypoparathyroidism | 4 ± 1 |
| 2 | Pseudohypoparathyroidism | 46,260 |
| 20 | Postoperative hypocalcemia | 18 ± 2 |
| 9 | Paget's disease of bone | 2 ± 1 |
| 2 | Sarcoidosis | 0 |
| 6 | Milk-Alkali | 0 |

(5) and others (6, 7). Jenifer Jowsey reported in 1967 (8) that increased bone resorption could be shown uniformly in bone biopsy specimens by her quantitative microradiography. These observations are of fundamental importance since the blood calcium level is low despite gross and microscopic roentgenographic evidence of increased bone breakdown. The reason for the hypocalcemia is therefore not clear. It is certainly not due to a lack of PTH-sensitive cyclic AMP in the bone. The elevated PTH levels in patients with pseudohypoparathyroidism fall promptly when the serum calcium is raised to normal by vitamin D therapy.

Hypocalcemia and hyperphosphatemia also occur, of course, in uremia. In such cases it is easy to exclude a simultaneous hypoparathyroidism, since the PTH levels are astronomical in uremia and low or nil in hypoparathyroidism. The high levels of uremia are not artifactually produced by any of the other abnormalities of uremia, since all of three patients with surgical hypoparathyroidism and coincidental uremia had zero levels. This point arises because some antisera are reportedly sensitive to urea (9) or to heparin (10). Our antisera are not affected by urea, heparin or by soluble collagen or fibrinolysin, which may be increased in uremia.

Another differentiation of great importance is the transient hypocalcemia and hyperphosphatemia which uniformly and nonspecifically follows all surgical procedures (11, 12). PTH levels are normal in these patients and not elevated as would be appropriate for the low serum calcium level, suggesting a transient depression of parathyroid response. The radioimmunoassay has made it possible for us to disprove hypoparathyroidism in several patients who had been mistakenly treated for hypoparathyroidism for years, in one case for 12 years. In these patients, the presence of normal PTH levels exonerated the parathyroid. Another condition where the serum PTH level is at or near zero is Paget's disease of bone. This finding in all of nine cases seems in accord with the evidence that Paget's disease is characterized by very rapid bone resorption, thereby acting as a constant calcium infusion. It parallels Cushard's recent observation of zero PTH levels in sarcoid where there is presumably also a constant inflow, in this case from the gut (13). Similar zero levels characterize the milk alkali syndrome as expected (Table III). Thus, all conditions of excess calcium inflow from bone or gut, except hyperparathyroidism itself, result in zero or near zero PTH levels.

It follows that PTH radioimmunoassay is of great value in the direct demonstration of primary hyperparathyroidism. The classic study of Berson and Yalow in 1966 (14) showed an overlap between the PTH levels in primary hyperparathyroidism and in normal subjects. Since hypercalcemia of causes other than hyperparathyroidism suppresses parathyroid function, the combination of hypercalcemia and measurable PTH is diagnostic of hyperparathyroidism. Figure 2 shows the serum calcium and PTH levels in 101 patients with parathyroid adenomas. In 14 of these, at least one of multiple determinations yielded zero values so that repeated examination is necessary to confirm the elevated PTH level. Others (15) have shown that the elevated levels in patients with parathyroid adenomas are suppressible by calcium infusions, thereby finally laying to rest the old myth of autonomy of parathyroid adenomas. Moreover, the relation of PTH to serum calcium level in patients with parathyroid adenomas yields a regression slope which is parallel to that of normal adults, but pitched higher. Therefore it is not surprising that, in some patients with adenomas, zero levels of PTH occur occasionally, perhaps because of calcium ingestion. We have been concerned that zero levels were found in 14 patients subsequently found to have parathyroid adenomas. These "false negatives" could have resulted from laboratory error, poor handling of serum, or possibly a

TABLE III

*Hyperparathyroidism*

| No. | Group | PTH µl eq/ml ± S.E. |
|---|---|---|
| 101 | Adenomas (1 cancer) | 39 ± 3 |
| 29 | Ectopic hyperparathyroidism | 27 ± 3 |
| 41 | Uremic children | |
| | 18 with osteodystrophy | 335 ± 53 |
| | 23 without osteodystrophy | 95 ± 12 |
| 50 | Stone-formers | 16 ± 2 |
| 94 | Breast cancer | 20 ± 2 |

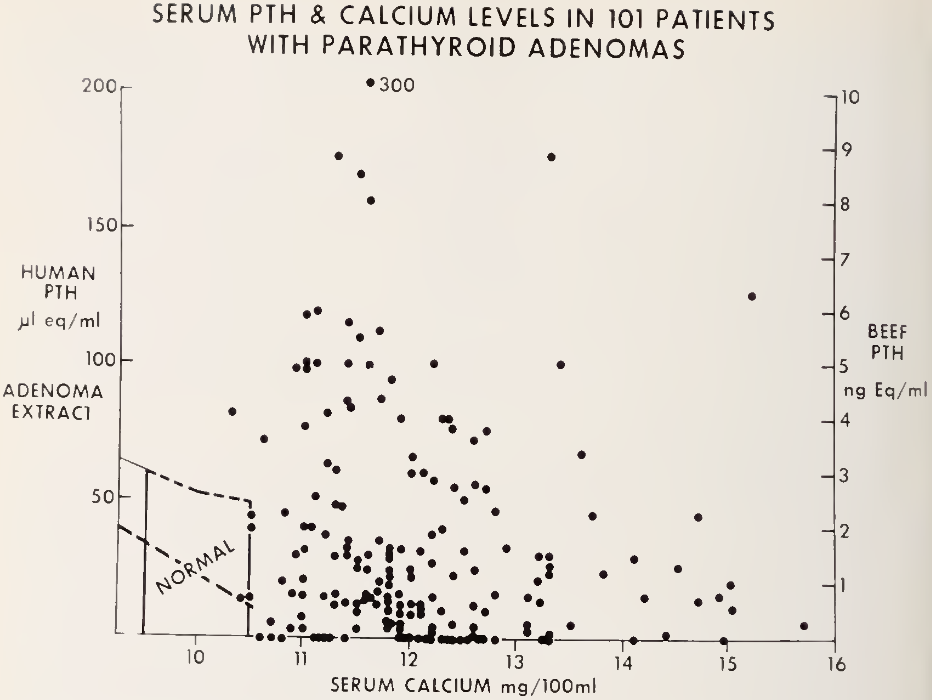

FIG. 2. Serum PTH and calcium levels in 101 patients with proved adenomas. Area of normal values previously reported in Am J Med 50:688, 1971. PTH reported as µl eq/ml. of extract of parathyroid adenoma referred to in Fig. 1.

molecular species of PTH not recognized by our antisera .In ten of the 14 cases the blood had been shipped from a distance, making inadequate preparation the most likely responsible factor. In the others, an unrecognized molecular species seems likely since the patient was studied in our laboratory during calcium deprivation.

"False positive" results, that is, diagnosis of hyperparathyroidism proved erroneous by negative operation, have occurred, to our knowledge, in three cases. It is noteworthy that all of these were in postmenopausal women. Yendt and Gagne (16) previously reported that serum calcium levels of women are lower than those of men. Werner et al (17) extended this observation by showing that the lower serum calcium levels of women are restricted to the reproductive years. This observation accords well with the time-honored action of estrogens to retard bone breakdown, recently shown directly by Jenifer Jowsey's sophisticated quantitative microradiography (18). It is well known that loss of this braking effect of estrogens on osteolysis is manifested both by postmenopausal osteoporosis and elevated serum phosphate levels (19). Less familiar is a rise of serum calcium levels in some women at the menopause. Estrogens lower the serum calcium to its premenopausal level in osteoporotic women (20). It seems likely that our "false positive" diagnoses, subsequently proved wrong at operation, resulted from erroneous evaluation of the serum calcium level as elevated in the presence of normal serum PTH values. This interpretation is borne out by the fact that serum phosphate levels were in the upper half of the normal range, and tubular reabsorption of phosphate was normal in all these women. This experience has made us quite cautious and in borderline cases we have waited as much as a year for the laboratory values to become clear cut.

The widespread use of routine laboratory screening has disclosed a large number of mild or borderline hypercalcemias, often with few or no symptoms. Contrary to previous experience (21), the established hypercalcemias have been explained most commonly by parathyroid adenoma, next by the milk-alkali syndrome (either iatrogenic or caused by proprietary antacid preparations), by thiazide treatment, and, fortunately, less often by malignancy. The clinical picture of hyperparathyroidism uncovered in this way differs from that seen in the past by the greater frequency of hypertension, diabetes, hyperuricemia, fatigue, and weakness. Familial occurrence is also more common than formerly; in these, recurrence within a year after removal of one or two parathyroid adenomas has also been more frequent. Unlike other reported series, primary parathyroid hyperplasia remains uncommon in our experience.

We have not identified any cases of normocalcemic hyperparathyroidism since the single case described from our laboratory by Eisenberg and Gotch in 1968 (22). The radioimmunoassay makes rather straightforward separation of hypercalcemia of nonparathyroid causes, for example milk-alkali syndrome, from primary hyperparathyroidism. Others have claimed that stone formers, most of whom have hypercalciuria, may represent a *forme fruste* of hyperparathyroidism and have even gone so far as to parathyroidectomize some (23). In 50 normocalcemic male stone-formers we found the serum calcium and PTH

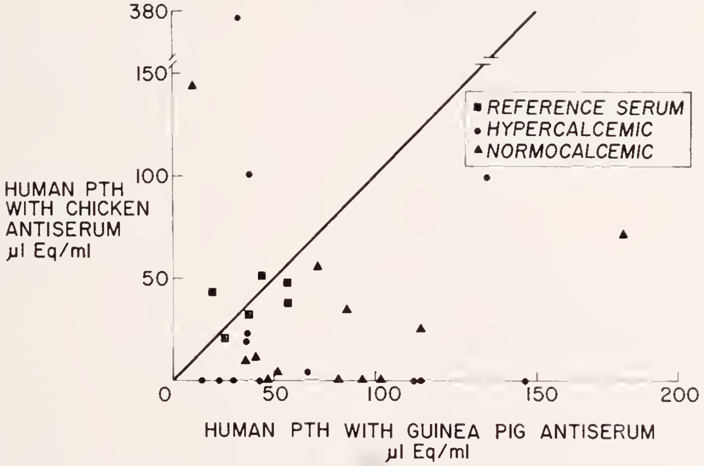

FIG. 3. Comparison of immunoreactive PTH in serum samples from 26 patients with malignancies other than breast cancer using two antiserums ■ = reference serum samples (two from patients with parathyroid adenomas and 4 from patients with severe uremia and renal osteodystrophy.

levels perfectly normal and have not, of course, subjected any such patients to parathyroidectomy. Nor has hyperparathyroidism been present in 94 patients with disseminated breast cancer. We have now had five cases of parathyroid adenoma in women with disseminated breast cancer and are aware of 11 others. These were readily diagnosed, either by radioimmunoassay or by the presence of hypophosphatemia and low TRP which are not features of breast cancer.

Hyperparathyroidism can also originate ectopically, that is, from cancer of organs other than the parathyroid gland. Berson and Yalow were the first to show elevated serum PTH levels in the absence of hypercalcemia in patients

TABLE IV

*PTH values in Nonuremic Children with Renal Disease*

| No. | Group | PTH µl eq/ml ± S.E. |
|---|---|---|
| 34 | Nephrosis | 45 ± 1 |
| 15 | Nephritis | 67 ± 3 |
| 10 | Familial nephritis | 47 ± 6 |
| 17 | Hematuria | 61 ± 25 |
| 9 | Lupus erythematosus | 47 ± 9 |
| 9 | Renal tubular acidosis | 46 ± 4 |
| 6 | Miscellaneous renal disease | 59 ± 11 |
| 100 | [Normal range ± 2 SD (0–89)] | |

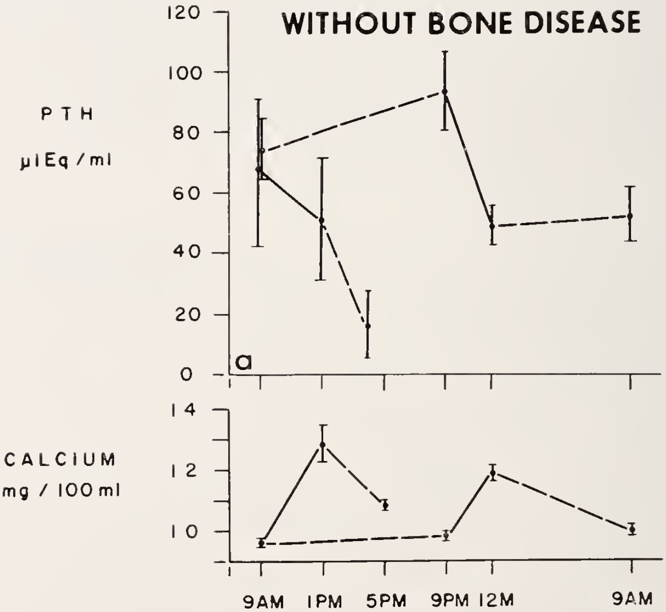

Fig. 4. A & B. Infusion of calcium in uremic children without (A) and with (B) renal osteodystrophy. Note rise in mean serum calcium and depression of mean serum PTH. Suppression was sustained at least 9 hours with nocturnal infusions in children without bone disease. In (B) suppression of PTH was not sustained but rebounded to preinfusion levels.

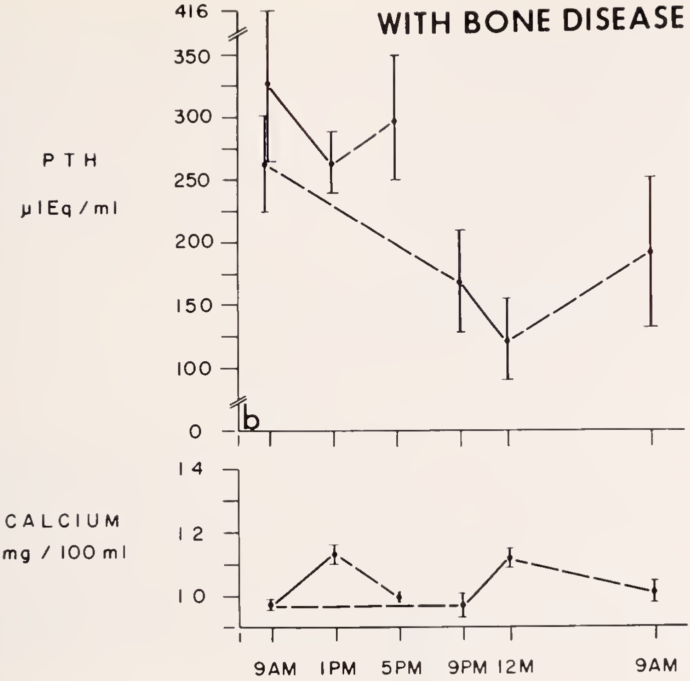

Fig. 4B.

with bronchogenic carcinoma (14). We have confirmed this phenomenon. Also in 14 of 26 selected patients with various malignancies (Fig. 3) (24), the peptide elaborated by these cancers differed from that secreted by normal, hyperplastic, or adenomatous glands, in that 11 were recognized by a guinea pig antiserum but not by a chicken antiserum, while in three the reverse was true. The Mayo group has recently confirmed immunologic abnormality of PTH and claimed differentiation of adenomatous from ectopic hyperparathyroidism by disproportionately low immunoreactive PTH for the serum calcium level (25). Our experience differs, perhaps because of use of a different antiserum. Just how early in the course of malignancy the abnormal hormone may be detectable and useful for early diagnosis remains to be ascertained.

From the relationship of serum calcium to PTH level, one expects elevated PTH levels in hypocalcemias not due to hypoparathyroidism. In uremia, these result both from impaired intestinal absorption of calcium and from hyperphosphatemia. Our data show that in renal diseases the PTH level actually rises before azotemia occurs. This is well demonstrated in various kidney diseases in a group of 100 children with chronic renal disease without uremia, where the serum creatinine was below 1.0 mg/100 ml. These children had nephrosis, nephritis, familial nephritis, hematuria, lupus erythematosus or renal tubular acidosis (Table IV). In long-standing uremia and osteodystrophy, as expected, very high levels are found.

It has been suggested that the secondary hyperparathyroidism of uremia may in some cases go on to an autonomous or tertiary stage (26). PTH radioimmunoassay permitted us to examine this thesis directly (27). Our evidence indicates strongly that the hyperparathyroidism of uremia is not autonomous: (1) the elevated PTH levels of uremic patients are readily suppressed by calcium infusion with a serum calcium rise of as little as 1.0 mg/100 ml (Fig. 4); (2) the elevated PTH levels are readily lowered by medical treatment with calcium salts or vitamin D which also produce bone healing (Fig. 5); and (3) the elevated PTH levels fall to normal after successful transplant with bone healing. Values are usually back to normal in 21 days in children but take longer in adults, perhaps because of involution of enormous masses of hyperplastic parathyroid tissue (Figs. 6 and 7). These data indicate that the hyperparathyroidism of uremia is secondary and readily controlled by appropriate medical therapy. We have had only one patient in whom a coincidental parathyroid adenoma was detected one year after successful renal transplant.

We have seen hypercalcemia in patients undergoing dialysis or following renal transplant. In some it has been traced to phosphate depletion due to aluminum antacids. The resulting hypophosphatemia mobilizes the soft tissue calcium-phosphate deposits which have been built up in the years of hyperphosphatemia. If this occurs during the period of parathyroid involution after successful renal transplant, hypercalcemia may be transiently associated with measurable PTH levels but in constantly diminishing amounts. It should be

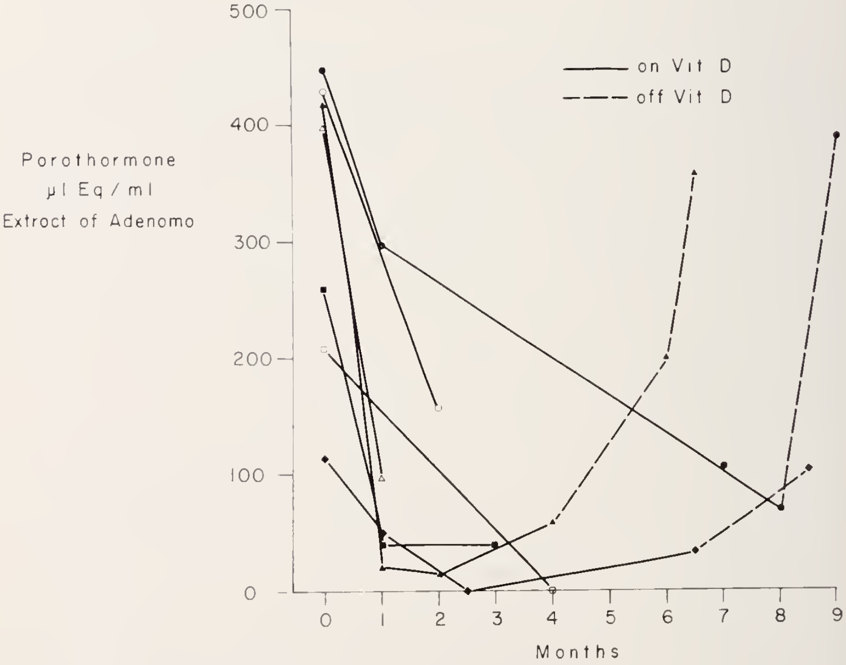

FIG. 5. PTH levels in uremic patients with renal osteodystrophy treated with vitamin D. Elevated levels decreased to normal ranges within 1–7 months and the bone healed. Note that when vitamin D was withdrawn the PTH levels rapidly rose to pretreatment levels.

recalled that parathyroid tissue may hypertrophy 200-fold in uremia, so that the amount of hormone released during involution is doubtless considerable.

Another condition characterized by secondary hyperparathyroidism, of course, is intestinal malabsorption with osteomalacia. The evidence for this relationship, dating back to Erdheim in 1907, (28) has been well documented. In 1956 Picchi et al reported that tubular reabsorption of phosphate (TRP) in patients with intestinal malabsorption, while almost always abnormal, could be either high or low (29). Low TRP correlated with roentgen evidence of subper-

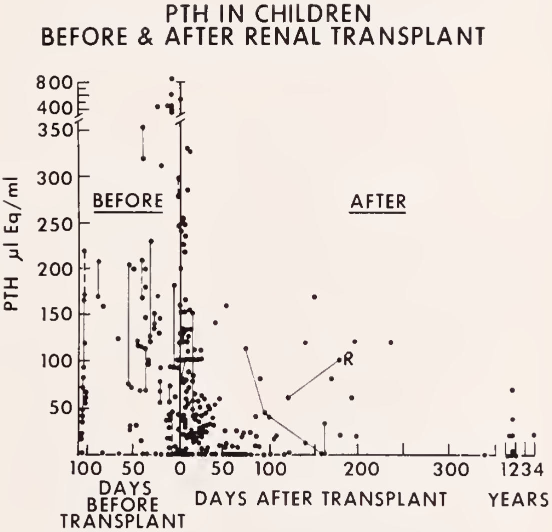

Fig. 6. Serum PTH levels of 25 uremic children before and after renal transplantation. The vertical lines represent suppression of PTH by calcium infusions and the broken lines by hemodialysis. The lines after transplant connect values from the same patient; where values became elevated, rejection (R) crisis was recognized.

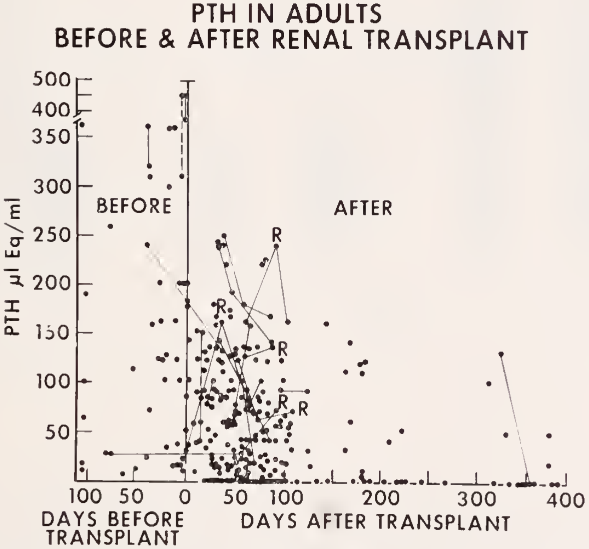

Fig. 7. Serum PTH levels of 50 uremic adults before and after renal transplantation. See Fig. 6.

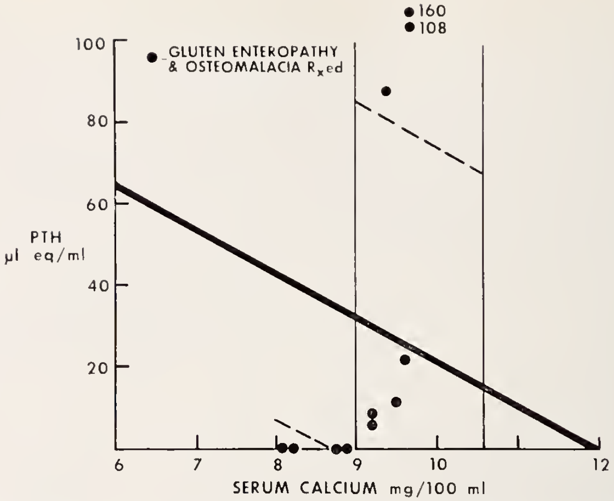

FIG. 8. Serum calcium and PTH levels in patients with gluten enteropathy and osteomalacia treated with vitamin D. Regression slope (dashed line ± 2 SD) derived from data on normals given oral or intravenous calcium gluconate.

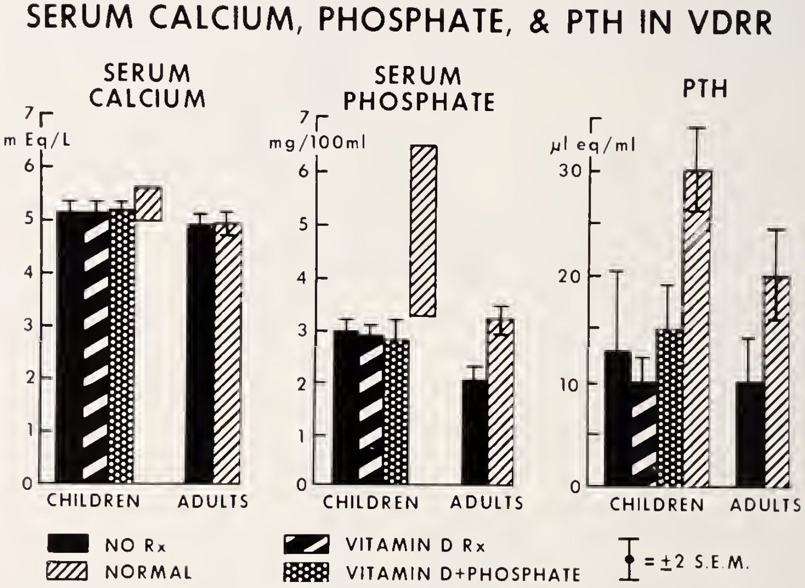

FIG. 9. Comparison of serum calcium, phosphate and PTH in children and adults with vitamin D-resistant rickets and in comparable age-matched controls. PTH is given as µl eq/ml. of a reference extract of a human parathyroid adenoma.

iosteal resorption of the phalangeal cortex, which is, of course, pathognomonic of hyperparathyroidism. Subsequently, Bernstein (30) proposed that parathyroid function might either be increased or alternatively, exhausted in patients with intestinal malabsorption. Direct measurement of PTH by radioimmunoassay supports this hypothesis, but surprisingly both high and low values are found in an alternating fashion in the same patients (Fig. 8). The secondary hyperparathyroidism of steatorrhea is characterized by low TRP which is easily turned off by calcium infusion (31). A similar renal tubular defect is found in patients with familial hypophosphatemic vitamin D-resistant rickets. In this condition also the low TRP can be raised by calcium infusion. The low TRP, however, is clearly not the result of secondary hyperparathyroidism in this particular condition, since direct measurement of PTH shows that it is not only not raised (32), but is actually low (Fig. 9) (33). Thus, the radioimmunoassay has opened a whole new approach to the study of the pathogenesis of familial vitamin D-resistant rickets.

In summary, the pioneering work of Berson and Yalow has now made it possible to measure PTH directly in human serum. Since this hormone, like other peptides, circulates in heterogeneous form, the sensitivity of each antiserum for the various molecular species and usual reference standards must be validated. Parathyroid secretion varies dynamically in response to altered serum calcium levels. Thus, in hypercalcemia of causes other than hyperparathyroidism, PTH is at or near zero levels. Values are also low in idiopathic or surgical hypoparathyroidism but are elevated in pseudohypoparathyroidism in response to hypocalcemia. The combination of high serum calcium and PTH levels occurs in patients with parathyroid ademonas or rarely, primary parathyroid hyperplasia, or in the syndrome arising ectopically from nonparathyroid cancers. False negative diagnoses are most commonly associated with poor technical preparation of the serum sample, but in some cases may represent a PTH molecule not detected by our antisera. In many cases of ectopic hyperparathyroidism, the hormone can be shown to be immunologically different from that found in normal, hyperplastic, or adenomatous hyperparathyroidism. Perhaps the single most important clinical application of the parathyroid immunoassay is the demonstration that the high levels found in the secondary hyperparathyroidism of uremia can be reduced by slight elevation of the serum calcium, by vitamin D treatment, or by correction of the uremia. Thus, parathyroidectomy is rarely, if ever, warranted in such patients, the secondary hyperparathyroidism responding to appropriate medical treatment. The hypercalcemia of nonparathyroid causes is readily differentiated by immunoassay. The situation in intestinal malabsorption is somewhat similar, but more complicated for reasons which need to be studied. In addition, the radioimmunoassay has raised new questions about the pathogenesis of familial, hypophosphatemic vitamin D-resistant rickets.

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# Chemical and Biologic Studies of Proparathyroid Hormone and Parathyroid Hormone: Analysis of Hormone Biosynthesis and Metabolism

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In the last three years there have been extremely rapid advances in our knowledge of the chemical properties of parathyroid hormone isolated from bovine, porcine, and human parathyroid tissue. The complete amino acid sequence of the major form of the bovine and of the porcine hormones has been determined (1–3). More recently, the sequence of the amino-terminal portion of the human hormone has been analyzed (4, 5). Synthesis of peptides consisting of the amino terminal 34 residues of the bovine and porcine hormone and numerous shorter sequences and analogues of the amino-terminal sequence of both molecules have permitted systematic analysis of the minimum structural features required for biologic activity (6).

The cumulative information concerning structure-activity relations of parathyroid hormone has assumed particular significance in physiologic and clinical studies of parathyroid hormone because of numerous reports (7–11) that indicate that the biosynthesis and metabolism of the endogenous hormone are complex. A biosynthetic precursor to human and bovine hormone has been identified and the structure of the bovine prohormone determined (12–16, 25). Multiple, chemically distinct forms of parathyroid hormone have been identified in the circulation. These findings have raised numerous questions concerning the regulation of parathyroid hormone production and the chemical and biologic properties of the active molecular species of hormone that interacts with receptor sites in bone and kidney. In addition, numerous difficulties in the assay of parathyroid hormone in blood have been encountered. These, in part, reflect the multiplicity of hormonal forms in the circulation.

## *I. Comparative Sequence of Parathyroid Hormone*

Parathyroid hormone has been isolated in the form of three homogeneous isohormones from the bovine species (17). Subsequently, the porcine hormone has also been purified (18). Our group was able to report in 1968 on results of partial sequence analysis of the amino-terminal portion of parathyroid hormone (19). However, it was appreciated at that time that the techniques used for isolation of hormone were still unsatisfactory. The purest material obtained still contained nonhormonal contaminants, constituting 10 percent to 30 percent

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of the total peptide. It was ultimately found that chromatography on carboxymethyl cellulose in 8 M urea was a satisfactory final step in isolation; this procedure results in the isolation of three different isohormones, free from each other and from nonhormonal contaminants (17). The major form of parathyroid hormone (bovine PTH-I) contains 84 amino acids and is devoid of cysteine and threonine. Both PTH II and III contain one mole of threonine and one less mole of valine than bovine PTH-I, but are otherwise similar in composition to PTH-I. Similar techniques were used for purification of porcine parathyroid hormone. Porcine parathyroid hormone also consists of 84 amino acids and lacks cysteine and threonine, but also lacks tyrosine and contains one rather than two residues of methionine. The complete covalent structure of porcine PTH and of bovine PTH-I has been determined (Fig. 1). Sequence analysis of the major form of the bovine hormone has also been independently determined in another laboratory (1); the results of the two groups are in complete agreement. Figure 1 shows the sequence of the bovine parathyroid hormone and, as well, positions where the porcine and bovine structures differ. Porcine parathyroid hormone has a different amino-terminal residue (serine instead of alanine), and also differs at two other positions (7 and 18) within the

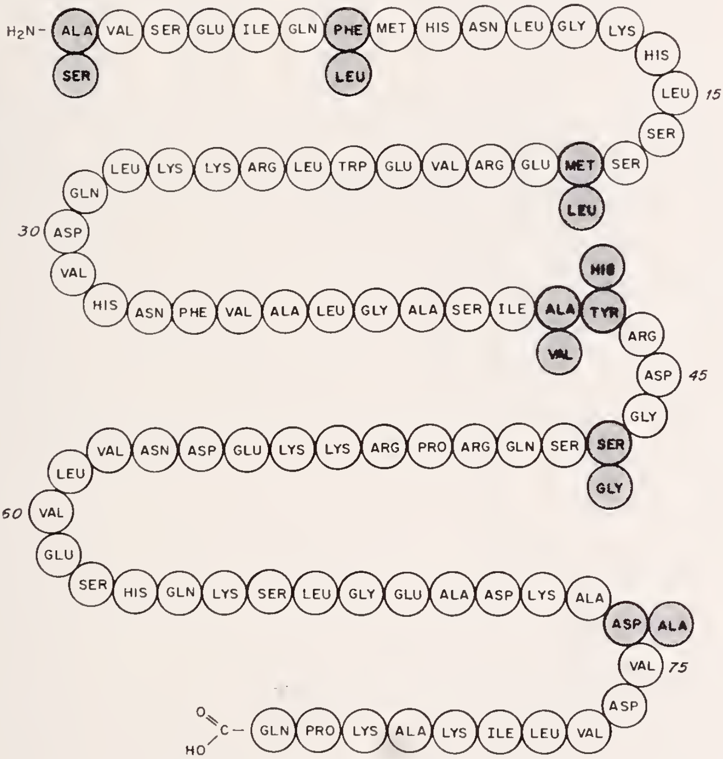
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FIG. 1. Amino acid sequence of bovine and porcine parathyroid hormones. The continuous linear sequence is the bovine hormone. Substitutions at 7 positions (1, 7, 18, 42, 43, 47, and 74) indicate the differences found in the porcine hormone.

biologically active amino-terminal region. The substitution at position 18 involves replacement of one of the two methionines present in bovine parathyroid hormone by leucine. The single tyrosine present at position 43 in bovine parathyroid is replaced by histidine in the porcine hormone. In all, the sequence of the bovine and porcine hormones differ in 7 positions (Figure 1).

Purified human parathyroid hormone has been available previously only in microgram quantities, insufficient for detailed studies of chemical properties (20). Recent improvements in extraction and isolation techniques, and the development of high-sensitivity methods for sequence determination have permitted us to obtain sufficient purified human parathyroid hormone to determine the amino acid sequence of the amino-terminal thirty-seven residues, a region including the biologically active portion of the molecule. A high-sensitivity procedure for automated Edman degradation in the protein sequenator was developed; the technique depends on the use of $^{35}S$ phenylisothiocyanate of high specific activity as the coupling agent. The resultant $^{35}S$ amino acid phenylthiohydantoins are identified by a two-dimensional thin-layer chromatographic system as well as by conventional gas and thin-layer chromatography. The newer technique improves the identification of each residue and, in addition, the yields of radioactivity at each cycle serve as an additional index of quantitative yield during degradation (5).

The structure we have found for the amino-terminal 37 residues of human parathyroid hormone differs from that of the bovine and porcine hormones, and from that cited in a recent report on the sequence of the human hormone (4). We find that human parathyroid hormone differs in sequence from the bovine hormone (Fig. 2) at positions 1, 7 and 16. The human and porcine molecules differ at positions 16 and 18. The asparagine found at residue 16 of the human peptide constitutes a sequence difference unique to human parathyroid hormone.

The recent report by Brewer et al (4) of the amino-terminal 34 residues of human parathyroid hormone differs from our structure. Brewer et al report residue 22 to be GLN, residue 28 to be LYS, and residue 30 to be LEU—all changes that would be unique to the human peptide. The structure proposed by Brewer et al contains changes that would constitute an increase of three in net charge of the molecule in the biologically active $NH_2$-terminal region, when compared to the bovine and porcine molecules and the structure we have found for human parathyroid hormone.

A difference in net charge of 3 in a sequence of nine residues would be expected to significantly alter immunologic reactivity. In many laboratories multiple antisera already have been developed by immunization with bovine and porcine hormone that are sensitive for the detection of native human hormone. Ultimately, comparative tests of immunologic reactivity in these immunoassay systems using native human hormone as standard and the synthetic peptides based on the structure proposed by Brewer et al versus that found by our group may help to resolve objectively the present discrepancy between the two laboratories. This will be quite important since it is obvious that immunoassays developed from synthetic human parathyroid polypeptides, to be useful

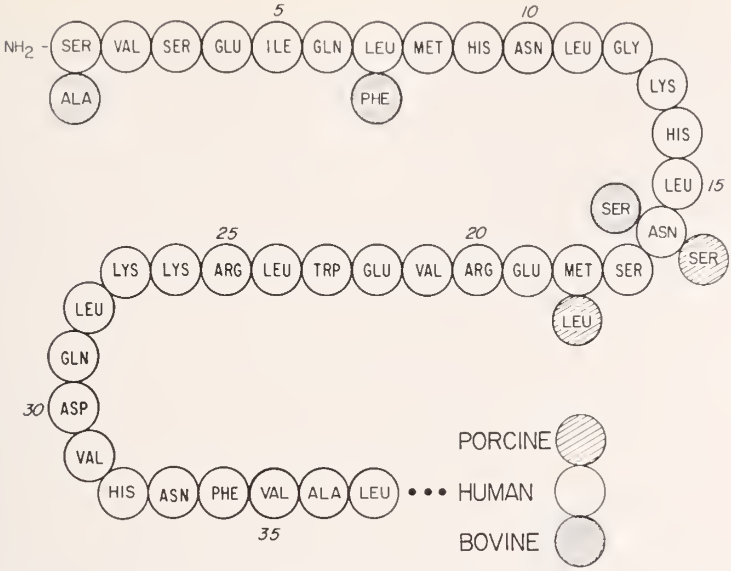

FIG. 2. Amino-terminal sequences of porcine, human, and bovine parathyroid hormones. The continuous linear sequence is the human hormone. Substitutions found in the porcine hormone are at positions 16 and 18 (cross-hatched circles); substitutions found in the bovine hormone are at positions 1, 7, and 16 (shaded circles).

in assay of endogenous human hormone, must more accurately measure human parathyroid hormone than existing immunoassays based on the bovine and porcine hormones. Assays based on synthetic peptides that differ from the true sequence of human hormone are more likely to confuse rather than clarify present difficulties with assay of human parathyroid hormone.

## *II. Structure-Activity Relations in Parathyroid Hormone: Peptide Synthesis*

Although the complete amino acid sequences of bovine and porcine parathyroid hormone are now known, there is still considerable uncertainty about the nature of the active molecular species of endogenous hormone. The chemical nature and biologic activity of the subfragments of the hormone that have been detected in the circulation are at present uncertain (8–11, 21). These findings concerning the complexity of hormone metabolism have provided an important impetus to analysis of the details of structure-activity relations in parathyroid hormone, particularly the biologic activity of hormone subfragments prepared by peptide synthesis.

Several fragments representing progressively shorter segments of the amino-terminal region of bovine parathyroid hormone have been prepared by solid-phase peptide synthesis and bioassayed in vitro (kidney adenyl cyclase) and in vivo (hypercalcemia assay) (6, 22). The synthetic amino-terminal 1–34 peptide was highly potent (80 percent on a molar basis of the the natural hormone in vitro and 130 per cent in vivo). Thus approximately two-thirds of the amino acid sequence found at the carboxyl end of the naturally occurring 84 residue peptide is not required for biologic activity. The high potency of the 1–34 peptide in the in vivo chick hypercalcemia assay further suggests that the carboxyl two-thirds of the native molecule must not play an important role in effective transport of the hormonal peptide to the bone receptor sites. It was found that deletion of the amino-terminal residue (alanine) resulted in marked decrease in activity and removal of the second residue (valine) at the amino terminus completely abolished activity in both assay systems. Essentially no deletion is possible, therefore, at the $NH_2$ terminus; greater shortening (7 residues) was tolerated at the carboxyl terminus. Peptide 1–27 was still active in the in vitro assay; deletion of residue 27 led to loss of biologic activity in both assays (6).

The results obtained with synthetic fragments of the bovine molecule seem applicable to structure-activity relations in human parathyroid hormone because of the close structural homologies between human and bovine hormones. The results of the recent studies (4, 5) of the sequence of the amino-terminal portion of the human hormone have shown only two differences between the structure of bovine and human hormone in the sequence region 2–27, substitution of leucine for phenylalanine at position 7 and asparagine for serine at position 16 (in addition, Brewer et al believe residue 22 is glutamine).

The important conclusion can, therefore, be stated that any circulating fragment of endogenous bovine parathyroid hormone in order to be active must represent a fragment from the amino-terminal portions of the hormone sequence no shorter at the carboxyl end than residue 27 and no shorter at the amino terminal end than residue number 2 (Fig. 3).

This information may prove to be useful in assessment of the physiologic significance of hormone metabolism and the chemical and biologic properties of the fragments of endogenous hormone recently detected in the circulation in man (8–11, 21) and in the bovine species (9).

## *III. Hormone Metabolism*

Several years ago, Berson and Yalow demonstrated that plasma parathyroid hormone is immunologically distinct from hormone extracted from human adenomas. This finding was of central importance in that, for the first time, it was seen that PTH in man was immunochemically heterogeneous. Their report led to widespread investigations of the character of plasma parathyroid hormone that, in turn, have led to important results concerning hormone biosynthesis and metabolism. More recently, it was shown in in vitro studies that the immunoreactive parathyroid hormone which accumulated in culture media when parathyroid glands were incubated in organ culture was of smaller molecular size than the stored 84 amino acid hormone. This led Arnaud et al (8) and Sherwood et al (21) to suggest that parathyroid hormone is cleaved in the parathyroid gland prior to secretion. This conclusion carried the further implication that the 84 amino acid peptide could not be the major endogenous, active form of the hormone in vivo since the intact hormone was not the principal

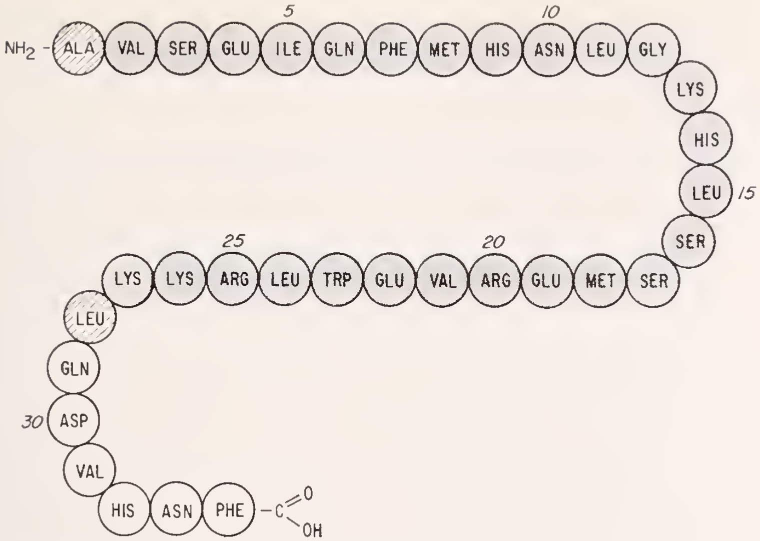

FIG. 3. The amino terminal 34 residues of bovine parathyroid hormone. The shaded region represents the minimum sequence length required for detectable biologic activity as determined in the in vitro and in vivo bioassays. Removal of either residue #1 or residue #28 (cross-hatched), although not causing complete inactivation, results in a synthetic peptide that gives a weak biologic response qualitatively different than that given by the native hormone.

secretory product. These important studies focused on the possibility that there were circulating hormonal fragments. However, it seemed necessary to evaluate the character of secreted hormone directly in vivo since the anoxia and tissue damage seen in organ cultures maintained for many hours in vitro might lead to artifacts (activation of proteases associated with tissue necrosis).

For these studies in vivo, particularly in order to definitely evaluate the immunochemical properties and size characteristics of endogenous circulating PTH in man, we developed radioimmunoassays using antisera that react specifically with defined regions of the hormone. The availability of synthetic hormonal fragments of BPTH have permitted us to characterize the antigenic recognition of several antisera. Antibody populations within these antisera are then selectively blocked by preincubation with excess concentrations of the appropriate fragments of the BPTH sequence. Antigenic recognition is thereby restricted to specific regions of the hormonal molecule. Use of these modified antisera enabled us to develop radioimmunoassays that measure specific regions of the hormone and apply them to a study of the immunochemical nature of endogenous PTH in man.

The studies in our laboratory have shown that in the peripheral circulation both of man and cow the immunoreactive parathyroid hormone consists largely of one or more fragments of the intact hormone. However, these studies have also suggested that most fragments result from cleavage after release of the hormone into the circulation and not from cleavage of the hormone in the gland prior to release. Release of predominately intact hormone was demonstrated by studies of the chemical properties of hormone released into the parathyroid venous effluent, by use of gel filtration, for size estimation, combined with use of multiple region specific antisera. It was found that most of the secreted hormone in man and cow is at least as large as the molecule extracted from glands.

Both the intact hormone and hormone fragment(s) are present in extremely low concentrations in plasma. It has not been feasible to test the biologic properties of the peptide fragments in unextracted or unconcentrated plasma. Therefore, efforts in our laboratory have concentrated on the application of the region-specific antibodies to characterize the fragments detected in blood. Since the minimum active sequence required for biologic activity has been identified, such immunochemical testing can be applied to determine whether any fragment in circulation has the structural features required for this activity. Thus far, studies with endogenous hormone have established, through gel filtration analysis of human peripheral plasma samples, that there is a large peak of immunoreactive material which elutes at a position corresponding to a molecular weight of approximately 7000. This fragment is detected using antisera that recognize antigenic determinants between residues 31 and 84, but not by antisera that recognize determinants amino-terminal to position 30. Since this large fragment lacks a portion of the critical amino-terminal sequence required for biologic activity, it must be biologically inactive.

Initial cleavage of the native peptide by an endopeptidase should result in formation of at least two fragments. With recognition of the large fragment, one might have expected to detect a second fragment of molecular weight approximately 2500. However, no smaller immunoreactive fragment has thus far been found in unconcentrated human blood. Failure to detect such a small fragment with present antisera might mean only that the region of the molecule constituting the fragment is not recognized by the antisera. It may also mean, however, that the smaller fragment is either largely destroyed at the time of cleavage or simply does not reenter the circulation; alternately, the fragment may be present in blood but is cleared rapidly from the circulation so that concentration of the small fragment is very low.

However, in preliminary animal studies in which large quantities of bovine hormone have been infused into the dog, a small fragment as well as the large fragment of approximately 7000 molecular weight has been identified. This small fragment is detected by three amino terminal antisera that fail to react with the large fragment. These studies indicate that the fragment might extend as far as residue number 33 and might be in the 3000–4000 molecular weight range. A recent report by Canterbury et al (28) working with concentrates of large quantities of human blood indicates a smaller fragment has been detected that stimulates renal cortical adenylcyclase activity. Their smaller peptide is stated to be approximately 4000 in molecular weight; the precise immunochemical properties of this peptide, that is, the region of the sequence of the intact hormone from which it arises, is at present unknown. Canterbury et al indicate that the active fragment constitutes 25 percent of total immunoreactivity in the concentrate; however, the concentration of the peptide in plasma prior to large-

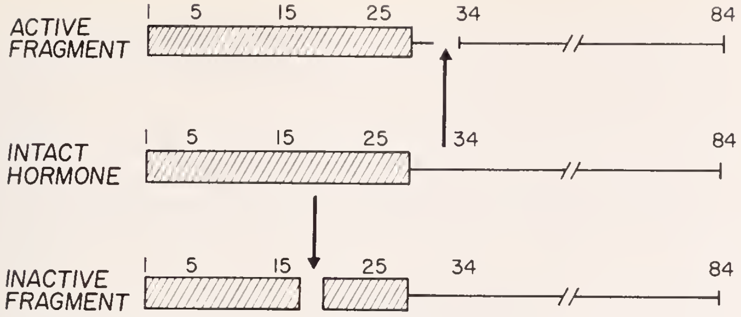

FIG. 4. Model of the two alternate metabolic cleavage sites of parathyroid hormone. The cross-hatched area indicates the minimal sequence required for biologic activity.

scale concentration is also unknown. It is not possible, at this time, to relate this interesting observation of Canterbury and Reiss to findings in our studies with region specific antisera and unconcentrated plasma samples.

Only when the exact site of cleavage of the secreted hormonal molecule is determined can the potential biologic significance of any fragment be unequivocally assessed. The synthetic studies permit the conclusion that a fragment of parathyroid hormone, in order to be biologically active, must consist of a continuous peptide sequence beginning with residue number two, valine, and extending as far as residue number twenty-seven, lysine (Fig. 3). As illustrated in Figure 4, if the cleavage occurs amino-terminal to position 27, both fragments produced by cleavage would be biologically inactive (based on structure-activity consideration) (Fig. 3). If cleavage occurs carboxy-terminal to position 27, the amino-terminal fragment resulting from the cleavage might have the structural requirements to be biologically active. Present investigations involving different approaches such as use of a series of antisera with more carefully defined recognition sites will help to identify more precisely the site of cleavage. If a smaller active fragment is produced, as suggested by our recent immunochemical studies and the observations of Canterbury et al, there would then seem to be two active molecular species, the intact hormone and a smaller amino-terminal fragment. The biologic value or physiologic significance of such reduplication of active hormonal species is not intuitively clear. Many interesting questions are posed concerning hormone metabolism. Peripheral cleavage might actually involve an activation process, rather than degradation and inactivation. The spectrum or duration of biological activity of a smaller fragment might differ from that of the intact molecule.

### *IV. Hormone Biosynthesis*

In addition to the hitherto unappreciated features of hormone metabolism, complexity has been uncovered with respect to the biosynthesis of the hormone. Biosynthesis of a true precursor (proparathyroid hormone) to human and bovine parathyroid has been demonstrated. It is important to distinguish conceptually between these recent studies concerning a prohormone and earlier studies in which the intact hormone itself was incorrectly termed a prohormone. At the time when it was believed that the intact hormone was not the form in which hormone was secreted but rather that hormonal fragments were the principal secretory product, intact hormone was described as a prohormone in the sense that it was the predecessor of the secreted form of the hormone. The true prohormone, larger in size than the 84 amino acid peptide, can be shown to be initially synthesized and then converted by enzymatic action into the hormone, which is stored in the gland until secretion. Hamilton and Cohn (12, 23) were the first to point to the probable existence of a prohormone. Formal kinetic proof of the presence of a biosynthetic precursor was demonstrated by Cohn et al (12) and by our group in collaboration with Kemper and Rich (14, 15). During incubation of tissue from bovine parathyroids and parathyroid adenomas, it has been shown that the proparathyroid hormone is labeled more rapidly than parathyroid hormone during incubation with $^{14}$C-amino acids and that the prohormone is progressively converted to the hormone. Studies using chemical and immunochemical techniques (14, 15, 24) have indicated that the hormone and prohormone are identical save for the presence of additional amino acids in the prohormone and that the prohormone specific sequence is added to the amino terminus of the parathyroid hormone sequence. Studies in progress to characterize chemically the prohormones from bovine and human tissue have met with initial success.

A biosynthetic precursor of bovine parathyroid hormone, proparathyroid hormone, has recently been isolated. It is not yet established whether larger precursor molecules exist. The sequence of the isolated pro-PTH has been determined (Fig. 5) (25).

The heavily basic hexapeptide sequence is added to the $NH_2$ terminus. An additional prohormone fraction was isolated in which the terminal lysine residue was absent (Fig. 5).

Synthetic fragments representing the prohormone hexapeptide sequence of parathyroid hormone (Fig. 3) and shorter analogs of this sequence have been prepared (26). Several biologic and immunologic tests have been performed to assess the properties of the prohormone (26, 27).

Biological activity of the pro-1–34 in vitro was much lower than that of the 1–34 peptide itself, 190 units/mg versus 5400 units/mg respectively, a molar ratio of approximately three percent (26). Somewhat higher activity of the pro 1–34 relative to the 1–34 peptide was detected in an in vivo assay; 1200 units/mg for pro-1–34 versus 7000 units/mg for 1–34, a molar potency ratio of approximately 20% (26). The results in vivo may reflect conversion of the pro-1–34 to 1–34 during transport through blood. Immunologic tests were used to compare cross-reactivity of (1) native pro-PTH to PTH and (2) pro 1–34 versus 1–34 Cross-reactivity of pro-PTH with an antiserum directed towards the carboxy-sequence 53–84 was approximately the same, whereas cross-reactivity of pro-PTH with antisera requiring all or part of the amino-sequence 14–30 was only one-fifth that of PTH. Cross-reactivity of pro-1–34 to 1–34 was one-tenth that of 1–34 (27). The results indicate that the presence on pro-PTH of the addi-

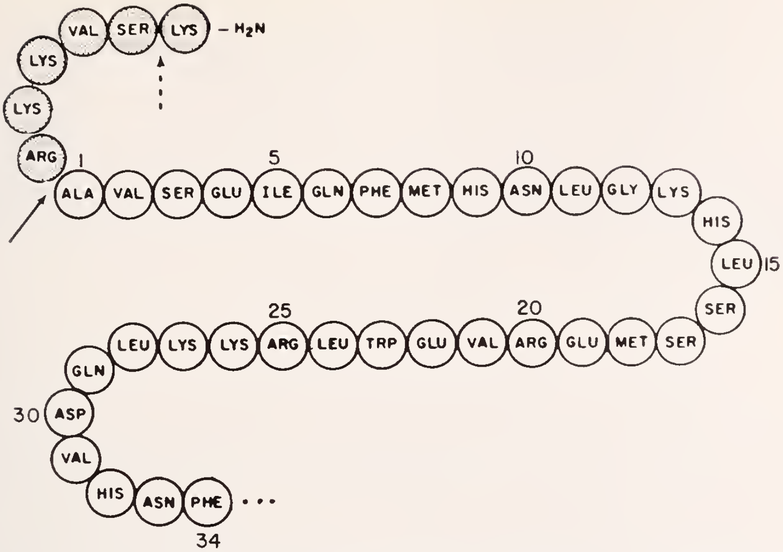

FIG. 5. The amino-terminal structure of bovine proparathyroid hormone. Unshaded residues 1–34 represent the amino-terminal sequence of bovine parathyroid hormone. The additional six amino-terminal residues found in the prohormone are shown as dotted circles. The continuous arrow shows the cleavage point for the conversion of prohormone to hormone; the dotted arrow represents a minor cleavage found in some prohormone preparations.

tional hexapeptide sequence produces conformational or other changes in the PTH molecule which impair binding both to antibodies and bioreceptors in target tissue.

Antisera have been raised against various synthetic peptides incorporating the bovine prohormone sequence. One antiserum in particular should prove to be very useful in physiologic studies of the prohormone as well as detection of the peptide in tissue extracts, tissue fluids, and in blood. The recognition site of this antiserum involves the sequence region at the site of attachment of the prohormone sequence to the hormone (27). The assay readily detects intact prohormone and synthetic peptides incorporating the prohormone sequence but not the hormone nor subfragments such as the prohormone hexapeptide sequence alone or the amino terminal peptides of the hormone, itself (27).

### V. *Summary: Implications of Present Knowledge Concerning Hormone Biosynthesis and Metabolism*

Thus, we now know that human and bovine parathyroid hormone undergo at least two specific cleavages from the point of initial cellular biosynthesis to ultimate disappearance from the circulation (Fig. 6). The first of these cleavages occurs in the cell when proparathyroid hormone (consisting of at least 90 amino acid residues) is converted to PTH (84 amino acids), the predominant species of the hormone that is secreted into the circulation. After secretion, the hormone then rapidly undergoes a second cleavage in the periphery; at least

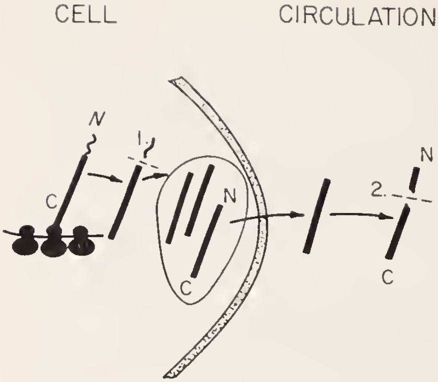

Fig. 6. Model of biosynthesis, secretion, and metabolism of parathyroid hormone in vivo.

one peak, eluting later than native hormone, corresponding to a biologically inactive fragment of molecular weight approximately 7000 daltons, and apparently a second fragment, are detected in the general circulation. Obviously, these specific cleavage steps could serve as points of metabolic control regulating both the amount of biologically active hormone available for secretion as well as the concentration of hormonally active peptides circulating and interacting with receptors in bone and kidney.

Many issues remain to be clarified about the biosynthesis and peripheral metabolism of parathyroid hormone. It is, for example, unknown at present whether the peripheral cleavage involves predominantly or initially a single site of attack within the molecule. It is also unknown whether the pattern of cleavage and subsequent clearance is the same in any one individual under different physiologic circumstances or from one individual to another within the human or other species. The similarity observed in the size and immunochemical characteristics of the large plasma hormone fragment detected in blood from one subject to another and the closely similar profiles seen on gel filtration of plasma samples from man and cow suggest, but by no means prove, considerable similarities in overall metabolism from one species or individual to the next.

Certain evidence (14, 15) suggests that in some parathyroid tumors the efficiency of cleavage of prohormone to hormone is less than in normal glands. The tumors may, in fact, secrete prohormone into the circulation; if this is true, such secretion adds further to the number of components in blood detectable by immunoassay. It seems possible that in the syndrome of pseudohyperparathyroidism due to ectopic production by cancer tissue of parathyroid hormone, the cleavage enzyme may be lacking in the randomly derepressed malignant tissue. If this is true, the product secreted by the nonparathyroid cancers could be exclusively prohormone; tests with prohormone specific antisera would then be of special diagnostic value. No direct tests of this possibility that prohormone is preferentially secreted by nonparathyroid cancers has been reported; however, some recent results concerning differences found between the patterns of immunoreactive hormone detected in blood from patients with primary hyperparathyroidism versus those with pseudohyperparathyroidism are compatible with this possibility (29, 30).

At the present time, it can be concluded that immunoassays of parathyroid hormone must be interpreted cautiously in view of the complex nature of circulating immunoreactive hormone. On the other hand, it is clear that immunoreactive hormone concentration in blood does correlate with chronic changes in parathyroid gland secretory activity and is, therefore, even at present, quite useful in numerous clinical applications (31–34).

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# Secondary Hyperparathyroidism

HOWARD RASMUSSEN, M.D., PH.D.

Being asked to write an article for this dedicatory volume is for me a singular honor. I was not a scientific collaborator or student of Dr. Solomon Berson but an intellectual and personal friend.

As other articles in this volume document, the radioimmunoassay technique has had a pervasive influence on clinical and experimental endocrinology. It has been of great value in a diagnostic sense, but even greater in an investigative one. It has allowed the formulation of whole new sets of questions in many different fields of enquiry. It is this aspect of Berson's contribution to the field of parathyroid physiology to which I should like to address myself. Others will discuss its more immediate contributions to parathyroid chemistry and the diagnosis of parathyroid disease. I should like to consider the similarities and differences seen in various types of secondary hyperparathyroidism in man.

## *Secondary Hyperparathyroidism*

A reliable immunoassay for parathyroid hormone has allowed us to replace supposition with fact in considering the nature and pathogenesis of secondary hyperparathyroidism in man. This is the case even though there are still important aspects of the metabolism of PTH which remain to be resolved, and some discrepancies from laboratory to laboratory in the species of hormone which is detected in human plasma.

The important causes of secondary hyperparathyroidism in man are listed in Table I. The most important group are the various forms of vitamin D deficiency and vitamin D dependency, those due to phosphate retention secondary to renal failure (with or without evidence of associated vitamin D deficiency). Less common but of considerable theoretical interest are pseudohypoparathyroidism, and cortisol excess due either to Cushing's disease or to the therapeutic use of these steroids. Whether calcium deficiency alone is a common cause of secondary hyperparathyroidism in man is a matter of debate. Fluorosis, on the other hand, is extremely uncommon in this country but does have a high incidence in certain parts of the globe, most notably in parts of India.

It is not yet clear whether the elevated concentration of PTH seen in patients with medullary carcinoma of the thyroid is primary or secondary (1). Medullary carcinoma of the thyroid is an endocrine tumor of the parafollicular or "C" cells found in this tissue. The rates of CT secretion and its plasma concentration are commonly increased in patients with this disease. It is a familial disease, and there is a very high incidence of functioning parathyroid adenomas in these patients. However, in a significant percentage of family members

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TABLE I

*A Classification of Secondary Hyperparathyroidism and Representative Changes in Plasma Electrolytes and Bone Remodeling in These States*

| Type | Plasma | | Bone Remodeling | | | | Clinical Bone Disease |
|---|---|---|---|---|---|---|---|
| | $Ca^{2+}$ | $HPO_4^{2-}$ | OPA* | R* | F* | Balance* | |
| Cortisol XS | N | N or ↓ | ↑ | ↑ | ↓ | ↓ | osteoporosis |
| Vitamin D deficiency-early | N | ↓ | ↑ | ↑ | ↓ | ↓ | osteoporosis with some osteomalacia |
| Vitamin D deficiency-late | ↓ | ↓ | ↓ | ↓ | ↓ | ↑ | osteomalacia |
| Renal phosphate retention | ↓ | ↑ | ↑ | ↑ | ↑ | ↓, N or ↑ | osteosclerosis and osteitis fibrosa cystica |
| Pseudohypoparathyroidism | ↓ | ↑ | ↑ | ↑ | ↑ | N or ↑ | normal bone mass |
| Medullary carcinoma of the thyroid | N | N or ↓ | ↓ | ↓ | ↓ | N | normal bone mass |
| Dietary calcium lack | N | N or ↓ | ↑ | ↑ | ↓ | ↓ | osteoporosis |
| Primary hyperparathyroidism | ↑ | ↓ | ↑ | ↑ | ↑ | N or ↓ | osteitis fibrosa cystica |

* OPA—osteoprogenitor cell activation; R—bone resorption; F—bone formation; Balance—the relative balance between formation and resorption.

parathyroid adenomas have been found in individuals without increased plasma CT values, i.e., the parathyroid adenoma appears to be a primary dysfunction of this tissue.

## *Control of PTH Secretion*

The one known regulator of the rate of PTH secretion is the concentration of ionized calcium in the blood plasma. It has been shown in experimental animals that the rate of secretion is an inverse function of plasma calcium concentration below a value of 10.5 mg per 100 ml (2, 3). An elevation of plasma inorganic phosphate concentration will also induce an increase in PTH secretion, but this is secondary to a phosphate-induced fall in plasma ionized calcium. Hence, all forms of secondary hyperparathyroidism share as a common pathogenic factor a decrease in plasma calcium ion concentration, but each of the diseases differs in the nature of the defect or defects which lead to hypocalcemia. Also, the effects which the elevated concentration of PTH has upon skeletal remodeling differ widely in these various states depending upon the factors which contribute to the initial hypocalcemia.

## *Mode of Action of PT*

Before discussing the similarities and differences in the various forms of secondary hyperparathyroidism, it is useful to consider briefly the mode of

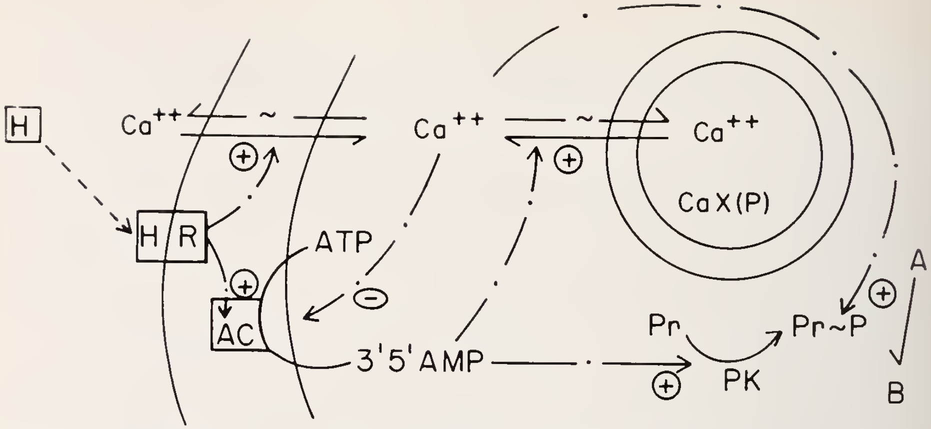

FIG. 1. A model of peptide hormone, and particularly PTH, action at the cellular level. The hormone (signal 1) leads to an activation of adenyl cyclase and stimulates the entry of calcium into the cell. The activation of cyclase leads to an increase in cyclic AMP (3′5′ AMP) within the cell which causes a net efflux of calcium from the mitochondrial calcium pool (CaX) to the cytosol. Cyclic AMP also activates a group of enzymes, protein kinase (PK). The combined effects of hormone at the cell membrane and 3′5′ AMP at the mitochondrial membrane lead to a rise in the [$Ca^{2+}$] in the cell cytosol. The rise in [$Ca^{2+}$] leads to an inhibition of adenyl cyclase, and an activation of other enzymes, some of which are the phospho-protein products of protein kinase activity.

action of PTH at both the cell and organ (bone) level (4). This hormone has at least two primary effects upon cell function (Fig. 1)—it increases the uptake of calcium into the cell, and it activates adenyl cyclase (5). The rise in cyclic 3′5′ AMP (cAMP) within the cell has at least two consequences: an activation of a class of enzymes known as protein kinase (6) and an increase in efflux of calcium from mitochondria (7). The combined effect of a stimulation of calcium uptake by the cell and of mitochondrial calcium efflux leads to a rise in the [$Ca^{2+}$] in the cell cytosol. This rise in [$Ca^{2+}$] leads to an inhibition of adenyl cyclase, and an activation or inhibition of enzymes, some of which are the phosphoprotein products of protein kinase activity. In addition, a rise in cytosolic [$Ca^{2+}$] increases the $Na^+$ and $K^+$ permeability of the plasma membrane of the cell.

In this system both calcium and cAMP act as second messengers in integrating cellular response to PTH. It is not yet clear whether they act in some sequential manner upon one or more metabolic pathways within the cell, or upon separate pathways. In all likelihood a mixture of both types of controls will be found. If they do act in part upon separate pathways, and it is recognized (Fig. 1) that their respective concentrations are mutually regulated within the cell, then factors which alter the relative ratios of cAMP and cytosolic [$Ca^{2+}$] will modify the cellular response to PTH (Fig. 2). In other words, this control system will exhibit a considerable degree of plasticity of response depending upon environmental circumstance.

Two factors which will alter this control system are the concentrations of $H^+$ and $HPO_4^{2-}$ in extracellular fluids (Fig. 2). This is so because a major pool of

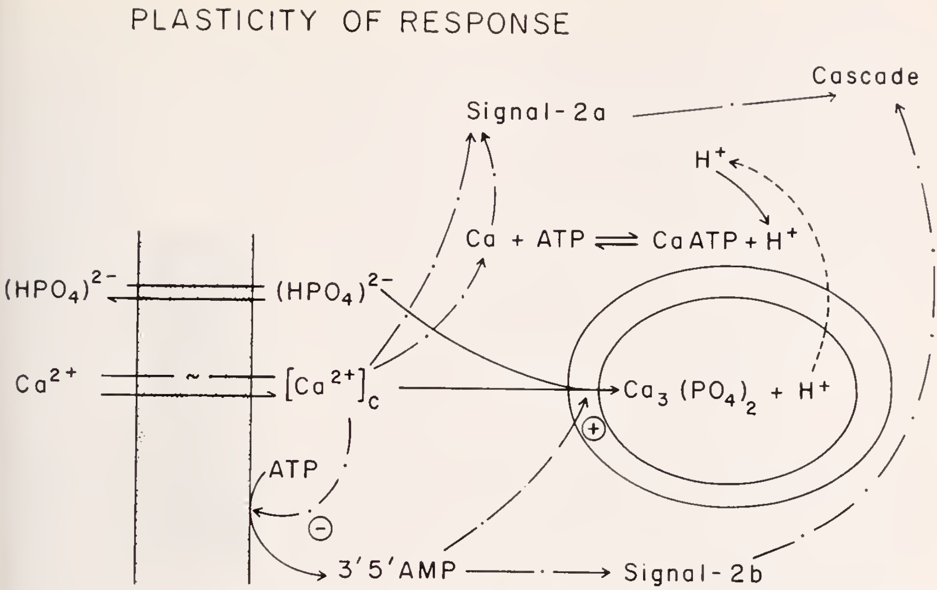

FIG. 2. The modulation of the basic cellular control system depicted in Figure 1 by $H^+$ and $HPO_4^{2-}$. There is a pool of insoluble $Ca_3(PO_4)_2$ within the mitochondria which is in rapid exchange with $Ca^{2+}$ and $HPO_4^{2-}$ within the cell cytosol and cell extracellular fluids. Also, when $Ca_3(PO_4)_2$ forms, $H^+$ are liberated. This system is one of the fundamental cell buffer systems for $Ca^{2+}$, $HPO_4^{2-}$ and $H^+$. See text for discussion.

cell calcium is that within the mitochondrial compartment of the cell and most of it exists as an insoluble complex of calcium phosphate which upon formation liberates $H^+$ $(3Ca^{2+} + 2HPO_4^{2-} \rightleftharpoons Ca_3(PO_4)_2 + 2H^+)$. This insoluble calcium pool is in rapid exchange with calcium, hydrogen and phosphate ions in cell cytosolic and extracellular fluid (7).

An increase in phosphate ion concentration in a cell stimulated by PTH leads to an increased movement of calcium into the mitochondrial pool (8). As a consequence, the $[Ca^{2+}]$ in the cell cytosol falls, adenyl cyclase inhibition is relieved and the [cAMP] increases (7). This, in turn, increases the rate of calcium efflux from mitochondria, thereby reestablishing a new steady state in which the $[Ca^{2+}]$ is lower, and the [cAMP] in the cell cytosol is higher. These changes are clearly an important component of the effect of phosphate upon bone cell function.

An increase in extracellular $[H^+]$ will lead to an increase in cytosolic calcium ion concentration, and thus to a decrease in [cAMP] because of a further inhibition of adenylcyclase by $Ca^{2+}$. In addition, an increase in $[H^+]$ inhibits directly the activation of adenylcyclase by PTH. The consequence is a cell in which the cytosolic $[Ca^{2+}]$ is higher and [cAMP] lower. These changes are an important component of the mechanisms by which $[H^+]$ changes bone cell function.

At the organ (bone) level, it has classically been taught that PTH stimulates bone resorption, and under some circumstances inhibits bone formation. How-

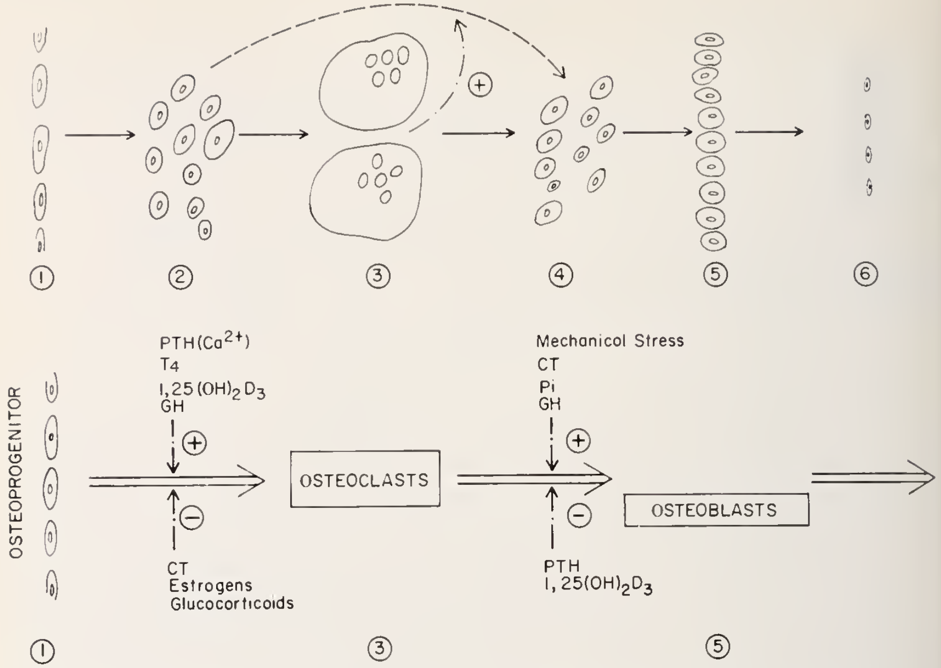

Fig. 3. The sequence of cellular events within a bone remodeling unit upon the endosteal bone surface and some of the factors which regulate this sequence. Osteoprogenitor cells ① are activated to undergo mitotic division to form daughter cells ② or preosteoclasts. These fuse to become osteoclasts ③ which, after carrying out the resorptive phase of skeletal remodeling, undergo fission to preosteoblasts ④. These in turn become osteoblasts ⑤ which carry out the formative phase of the remodeling cycle and in the process become incorporated into bone as osteocytes.

ever, the most basic feature of endosteal bone cell remodeling must be understood to appreciate the mode of action of this hormone. The most distinctive feature of bone remodeling is that it is a sequential process (9) at any given area of bone surface (Fig. 3). The bone surface is normally covered by a thin layer of mesenchymal cells. Upon some signal, which is in part hormonal in nature (see below), a group of these cells are activated to undergo several waves of mitotic division. These osteoprogenitor cells become preosteoclasts which fuse to become osteoclasts, the cells of bone surface resorption (10). After carrying out the resorptive phase of surface remodeling, these cells undergo fission to mononucleated cells which become, in turn, preosteoblasts and then osteoblasts, the bone-forming cells (10). These carry out the formative phase of bone remodeling and then become either osteocytes or revert to surface mesenchymal cells. This sequence of activation → resorption → formation defines the lifetime of a bone remodeling unit (9). The maintenance of skeletal homeostasis depends upon the sum of remodeling events in all the bone remodeling units. Skeletal balance, that is, formation equals resorption, can be maintained under conditions of very high or very low rates of bone turnover.

This system can be characterized in two ways: 1) the total rate of remodeling or rate of activation of new remodeling units; and 2) the balance between resorption and formation (Table I). In an operational sense the balance between formation and resorption depends upon the size of the osteoclast and osteoblast cell pools, and upon the activity of the individual cells within each pool (Fig. 3). In order to enhance net bone resorption PTH could act in one of several ways: 1) enhance the activity of individual osteoclasts; 2) increase the size of the osteoclast pool either by inhibiting the flow of osteoclasts to osteoblasts; 3) decrease the activity of individual osteoblasts; or 4) decrease the size of the osteoblast pool either by inhibiting the flow of osteoclasts to osteoblasts or by increasing the flow of osteoblasts to osteocytes. There is evidence that the hormone exerts all four types of actions. However, it is important to distinguish between the acute and the chronic effects of PTH administration.

In the acute phase of experimental hyperparathyroidism, osteoclastic activity is enhanced, and osteoblastic activity inhibited, and the rate of modulation of osteoclasts to osteoblasts reduced. However, with time more and more osteoprogenitor cells are activated, and the rate of fusion of new osteoclasts increases. As the size of the osteoclast pool increases the rate of modulation of osteoclasts to osteoblasts increases. As a consequence, the size of the osteoblast pool increases, and there is a secondary rise in rate of bone formation. Hence, in the chronic phase of experimental hyperparathyroidism the rates of both bone resorption and bone formation have increased. In mild clinical hyperparathyroidism the net increase in formation is usually sufficient to restore a net balance between these two activities. In the more severe form of the disease, this usually is not the case, and most often there is a net negative skeletal balance, that is, the bone removed during the resorptive phase is not completely replaced during the subsequent formative phase. However, even this imbalance is not a necessary consequence of excess PTH because the relative magnitudes of the two phases of remodeling activities can be influenced by independent factors, for example, growth hormone and inorganic phosphate.

The clearest example of this is the effect of inorganic phosphate upon calcium balance and calcium metabolism in patients with primary hyperparathyroidism as first described by Albright (11). Administration of phosphate leads to elevation of plasma phosphate concentration, a reduction of plasma calcium concentration, and a less negative or even a positive calcium balance. At the level of bone cells, phosphate enhances the modulation of osteoclasts to osteoblasts, and stimulates the activity of individual osteoblasts. Both of these effects are opposite to that of PTH. However, phosphate does not normally appear to reduce greatly the effect of PTH upon osteoprogenitor cell activation (Fig. 3).

Having defined the effects of PTH at both the cell and organ level in uncomplicated primary hyperparathyroidism, it is now possible to return to a consideration of the various forms of secondary hyperparathyroidism and consider how the effects of excessive PTH upon bone cell function are modified as a consequence of other ionic or hormonal factors.

### *Vitamin D Deficiency and Cortisol Excess*

It has often been held that the effects of excess adrenal corticosteroids upon calcium metabolism are similar to a deficiency of vitamin D, that is, that cortisol acts as a vitamin D antagonist (12). However, this is only true at a superficial level of analysis. There is a crucial difference in the two states. This difference is their consequences upon bone cell function.

In the case either of vitamin D deficiency or cortisol excess, significant factors are an inhibition of intestinal calcium absorption and of renal tubular calcium reabsorption. In spite of these similarities at the level of kidney and intestine, vitamin D deficiency and cortisol excess lead to quite different consequences at the level of bone. Cortisol augments the effect of PTH in causing an imbalance between bone formation and resorption in favor of the latter (13). As a consequence, bone mass declines, that is, osteoporosis develops (Table I), and this negative skeletal balance plays an important role in maintaining plasma calcium and phosphate concentrations at nearly normal values in this form of secondary hyperparathyroidism. In a sense then, the secondary hyperparathyroidism of cortisol excess is a normocalcemic secondary hyperparathyroidism; the most serious consequence of which is osteoporosis.

Vitamin D deficiency leads initially to a very similar change in bone, a type of secondary hyperparathyroidism associated with bone loss. During this phase of the disease, plasma calcium concentration is usually near normal although plasma phosphate concentration is reduced (Table I). However, eventually D deficiency leads to a specific lack of calcification of newly formed bone matrix. The progressive formation of osteoid, which cannot be resorbed, and which covers the mineralized bone so that bone resorption then fails to supply sufficient bone mineral to maintain mineral homeostasis. When this occurs, hypocalcemia develops. Severe or advanced vitamin D deficiency leads to hypocalcemic and hypophosphatemic secondary hyperparathyroidism (Table I).

The disease is clearly different from the secondary hyperparathyroidism of cortisol excess. It is different in two ways. In order to understand these differences, it is necessary to digress for a moment to consider the relationships between PTH and vitamin D.

One of the most important recent discoveries in the field of calcium metabolism is that vitamin D undergoes metabolic transformations before exerting its biologic effects (14). The initial step is the conversion of vitamin $D_3$ to 25-hydroxyvitamin $D_3$ ($25(OH)D_3$). The second step, which takes place exclusively in the kidney, is the conversion of $25(OH)D_3$ to 1,25-dihydroxy-vitamin $D_3$ ($1,25(OH)_2D_3$) (15). This latter step is the point of major control in this metabolic sequence. The product of this reaction, $1,25(OH)_2D_3$, is thought to be the metabolite of vitamin D which is responsible for the biological effects of vitamin D upon both intestine and bone.

Parathyroid hormone is related to this metabolic sequence in two ways (Fig. 4). It regulates the rate of conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$. An increase in PTH causes an increase in $1,25(OH)_2D_3$ synthesis. The second

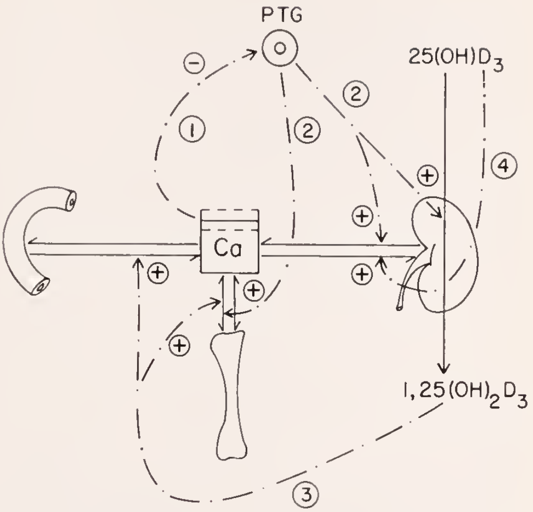

FIG. 4. A schematic representation of the role of PTH in plasma calcium homeostasis. When plasma calcium concentration falls ① it leads to an increase in the rate of PTH secretion ② from the parathyroid glands (PTG). The secreted PTH ② acts directly upon two organs, bone and kidney. Its acute effect upon bone is to enhance resorption and decrease formation causing a net shift of calcium into the extracellular fluid compartment. At the level of the kidney, PTH induces the renal tubular reabsorption of $Ca^{2+}$ which also causes a retention of calcium within the extracellular fluid compartment. PTH also stimulates the renal conversion of 25(OH)$D_3$ to 1,25$(OH)_2D_3$. The 1,25$(OH)_2D_3$ acts synergistically with PTH at the bone level, and also enhances the absorption of calcium from the gastrointestinal tract. The control of 1,25$(OH)_2D_3$ synthesis in the kidney is a means by which the activities of the three organs are coordinated in this homeostatic control system.

relationship is that the presence of 1,25$(OH)_2D_3$ is necessary for PTH to exert its characteristic effects upon bone, particularly its effects upon osteoprogenitor cell activation.

Returning to a discussion of the secondary hyperparathyroidisms of cortisol excess and vitamin D deficiency, the most important differences involve activities at the level of intestine and bone. When cortisol is given, there is a compensatory increase in PTH which stimulates 1,25$(OH)_2D_3$ synthesis, which, in turn, counteracts to an extent the inhibitory effects of cortisol upon intestinal calcium transport and which allows PTH to exert its effects upon bone cells. In contrast, in vitamin D deficiency the secondary rise in PTH concentration is ineffective in enhancing intestinal calcium absorption or bone cell activation because 1,25$(OH)_2D_3$ synthesis does not occur due to a lack of substrate.

Upon the basis of these facts and the observations that vitamin D lack leads to a marked decrease in the size of the mitochondrial calcium pool within cells, it is possible to relate the effects of vitamin D to the model of cellular events following PTH action (Fig. 5). The major deficiency in the D-deficient cell is a decrease in the concentration of calcium in the extracellular fluids and a diminished calcium content in the mitochondrial pool. Both of these calcium pools are involved in the cellular effects of PTH. This means that in the D-deficient cell when PTH acts it does not cause a very significant rise in the $[Ca^{2+}]$ in the cell cytosol as it does in the normal cell (Fig. 5). Because this increase in the $[Ca^{2+}]$ in the cell cytosol is one of the important intracellular signals, or second

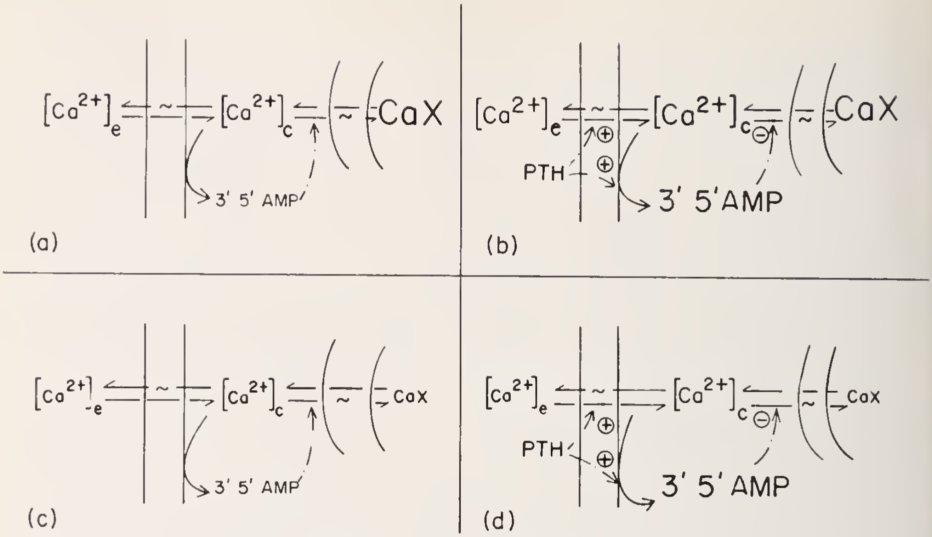

Fig. 5. A model of the mode of action of vitamin D upon cell function in relation to the model of PTH action depicted in Figure 1. The major deficiency in the D-deficient cell (c) is a marked depletion of the mitochondrial calcium pool (CaX) in comparison with the normal cell (a). Thus when PTH acts upon the normal cell (b) the rise in 3′5′ AMP within the cell leads to a marked rise in the $[Ca^{2+}]_c$ within the cell cytosol, but in the D-deficient cell (a) the rise is less marked in spite of the fact that the increase in 3′5′ AMP concentration is just as great or greater.

messengers, after PTH action, cell activation does not take place in the D-deficient animal even though PTH has activated adenyl cyclase.

If this model is correct, it predicts that the administration of calcium to a D-deficient organism sufficient to raise the plasma calcium ion concentration toward normal, but not to normal (because this would reduce PTH concentrations to normal values), should lead to an increase in bone cell activation and an increase in osteoclast number upon endosteal bone surfaces. This prediction has been verified (15).

### *Phosphate Retention and Secondary Hyperparathyroidism*

Another major cause of secondary hyperparathyroidism is phosphate retention (16) secondary to renal disease (Table I). This is often complicated by a concomitant deficiency of vitamin D. However, in some patients with chronic renal disease the major factor in inducing hyperparathyroidism is phosphate retention. In these patients the bone disease which develops is often characterized as hyperparathyroidism with osteosclerosis (Table I). There is a net increase in new bone formation because, as discussed previously, phosphate enhances the modulation of osteoclasts to osteoblasts. The combination of hyperparathyroidism and hyperphosphatemia leads to an increase in rate of total skeletal remodeling and a net balance of remodeling events in favor of formation (Fig. 3).

At the cellular level, these effects of phosphate can be related to its effects upon cell calcium metabolism (Fig. 2). An increase in phosphate causes a shift of calcium into mitochondria thereby reducing cytosolic [$Ca^{2+}$].

A rare but most interesting example of this relationship is pseudohyperparathyroidism. This is a condition in which there is a lack of the normal renal response to PTH. As a consequence, plasma calcium falls and plasma phosphate concentration rises. The resulting hypocalcemia stimulates PTH secretion. This increase in hormone is ineffective in altering renal function but does act upon bone. However, severe and progressive loss of skeletal mass does not occur. This is due to the fact that the high plasma phosphate concentration stimulates the conversion of osteoclasts to osteoblasts and stimulates the activity of these osteoblasts so that there is no imbalance between formation and resorption even though the overall rate of bone turnover has increased (Table I).

### *Medullary Carcinoma and Secondary Hyperparathyroidism*

Assuming for the moment that, at least in some patients with medullary carcinoma (1), the increased concentrations of PTH in plasma are secondary to the increased secretion of calcitonin seen in this disease, it is worth analyzing the nature of this secondary hyperparathyroidism.

The effects of CT upon bone cell function are opposite to those of PTH (4). CT causes an increase in the rate of modulation of osteoclasts to osteoblasts (10). As a consequence, there is a transitory increase in net bone formation. However, CT also causes an inhibition of osteoprogenitor cell activation which leads to a decrease in osteoclast pool size followed by a reduction in osteoblast pool size. Thus, in medullary carcinoma the rates both of bone resorption and formation are greatly reduced, but there is no imbalance between these two activities. This means that chronic excessive CT will not produce hypocalcemia due to an imbalance between bone remodeling activities. However, CT has at least two other effects which lead to hypocalcemia: 1) an inhibition of the renal synthesis of $1,25(OH)_2D_3$ (17); and 2) an increase in urinary calcium excretion (18). These lead to a hypocalcemia which in turn activates PTH secretion. This rise in plasma PTH concentration is sufficient to counteract the renal effects of CT so that plasma calcium concentration is nearly normal, but this level of PTH is insufficient to overcome the inhibitory effect of CT upon osteoprogenitor cell activation. The secondary hyperparathyroidism in this condition is of the normocalcemic variety, but is distinguished particularly by the very low rates of bone remodeling (Table I).

## Conclusion

Secondary hyperparathyroidism is a response to a fall in plasma calcium concentration. It occurs in a variety of disease states in man (Table I) including cortisol excess, vitamin D deficiency, renal phosphate retention, pseudohypoparathyroidism, and medullary carcinoma of the thyroid gland in which excessive calcitonin secretion takes place. The effects of the elevated concentrations of plasma PTH upon bone cell function and skeletal remodeling are quite dissimilar depending upon the nature of the factor or factors which induced the secondary hyperparathyroidism. Of particular importance in modifying the cellular response to PTH are vitamin D, inorganic phosphate, CT and adrenal glucocorticoids. Depending upon the presence of these ionic and hormonal factors, secondary hyperparathyroidism can be associated with a greatly enhanced or reduced rate of skeletal remodeling—with either a balance of remodeling activities or an imbalance in favor of either resorption or formation. These differences are explicable in terms of a sequential model of bone cell function. The importance of explaining these differences and validating this model lies in the hope that it can serve as a logical basis for therapeutic approaches to metabolic bone diseases in man.

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# Labeled Albumin: Some Recent Contributions to Current Concepts concerning Regulation of Protein Metabolism

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It was 20 years ago that I first began to work at the Veterans Administration Hospital in the Radioisotope Service under the direction of Dr. Solomon Berson. This experience was to grow into a lifelong friendship, one in which I took great pleasure. Dr. Berson was a man of so much warmth, so much giving and so many talents that his contributions will span periods of time greatly in excess of the shortened life span that his own career encompassed. It was while working with him that I became interested in serum albumin metabolism and distribution. Many basic questions in the field of serum protein metabolism are answered in some of Dr. Berson's early work. The use of iodinated albumin for the study of endogenous albumin metabolism has become rather routine in many institutions, but the precautions laid down by Dr. Berson in the early studies have paved the way for this work. Most of the studies utilizing iodinated albumin have to depend on accurate measurements of the intravascular volumes for the space of distribution of the iodinated protein. It was in 1952 (1) that Dr. Berson observed that iodinated albumin really did not leak very rapidly from the circulation, with the loss of labeled albumin and red cells being less than one percent per 15 minutes during the first few hours. Thus, corrections for this loss are less than combined errors of pipetting and counting. For all purposes a single point obtained a few minutes after injection should suffice for the determination of the intravascular distribution of iodinated albumin. This observation has been confirmed repeatedly and during the first two hours usually less than six to seven percent of the iodinated albumin is lost from the intravascular pool even in patients with marked ascites or congestive heart failure with edema (2–5). Following the intravascular equilibration, albumin does achieve an eventual space of distribution such that in normal individuals, 60 to 65 percent of exchangeable albumin is located in extravascular sites. This equilibration takes place at rates varying between a few hours or more than a day. Eventually distribution equilibrium will be achieved such that the tracer dose of radioactive albumin will be in essential equilibrium with the endogenous albumin in the various compartments. Obviously, since albumin is synthesized in the liver and deposited directly into plasma (6), the specific activity of the plasma pool will always be slightly lower than the specific activity of albumin located in extravascular sites. As long as albumin degradation is relatively slow in comparison to albumin distribution, this difference in specific activity between plasma and extra plasma pools will not cause significant errors in the

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measurements of exchangeable albumin. However, if loss is rapid, then albumin returning from extravascular sites via the lymphatic system will not have a chance to equilibrate prior to the loss of significant quantities of labeled albumin from the intravascular pool (7). In situations where rapid changes in the exchangeable albumin pool may be taking place, measurements of the plasma disappearance curve alone will not give accurate estimates of the fractional rate of albumin degradation. These concepts were embodied in Dr. Berson's studies of the kinetics of albumin metabolism and distribution and have served as the basis for the study of serum protein metabolism.

The concept of the metabolic clearance technique has added an important parameter to the study of the absolute rates of albumin degradation. This concept first devised by Dr. Berson (8), relates the excretion of iodine from the products of albumin degradation to the plasma concentration of $I^{131}$-labeled albumin during the period of time that the urinary excretion is monitored. Assuming that the plasma albumin level remains constant during this time, the product of clearance and the serum albumin level will result in values for the absolute amounts of albumin degraded per day. This technique may be employed during nonsteady state situations. Marked increments in albumin degradation have been observed during the administration of thyroid and cortisone hormones (9, 10), significant decreases in albumin degradation have been noted in the presence of exogenously administered dextran, gamma globulin, or endogenously produced hypergammaglobulinemia (11). While it was not possible to obtain specfic values for the fractional rates of degradation during these rapidly changing states, the absolute value for total daily albumin degradation during the experimental period or the stress period would permit an estimate of net synthesis. A second injection of albumin $I^{131}$ following the attainment of a new steady state permits the reassessment of the exchangeable albumin pool. Thus, if the exchangeable albumin pool was known before and after the attainment of new steady state and the absolute rates of degradation determined during the experimental period, then a change in the net pool size would be indicative of a net change in synthesis. Employing this technique it was possible to show that albumin degradation and synthesis were not interdependent (12); that the body prevented the accumulation of excess intravascular colloid, during infusion of colloidal substances other than albumin, by altering rates of albumin synthesis (11). However, when serum or albumin were infused, the prevention of the increment of plasma colloid occurred by means of increasing the fractional and absolute rates of albumin degradation. Net albumin synthesis remained unchanged (12). These observations suggested that albumin degradation was mass-related and that as the exchangeable albumin pool fell, either due to a defect of synthesis and/or to increased loss from the body, the absolute amount of albumin degraded decreased. Unless both the fractional and absolute degradative rates are measured, marked changes in albumin degradation will be minimized or missed completely.

The degradative mechanism, which is still unknown, is respondent in some fashion to the total mass of circulating and/or exchangeable albumin. The degradative compartment or compartments are located in close approximation to the plasma and in rapid equilibration with both plasma and extravascular albumin pools (13, 14). While no organ has been shown to play an important role in albumin degradation in health, both the kidney and the intestinal tract appeared to have significant effects on albumin degradation and loss in disease states (15–21). In the absence of tubular disease, small protein molecules, filtered at the glomerulus, are absorbed and catabolized by the tubular mechanism. Thus endogenous metabolism continues. In the presence of tubular disease proteinuria ensues, but the tubular degradative mass is less. Thus, endogenous degradation would decrease while the total degradative process (loss plus degradation) would increase. In severe uremia where the functioning mass of the kidney is destroyed, endogenous degradation as well as loss may prove insignificant.

In disease of the intestinal tract, significant amounts of albumin may be lost either through disease of the mucosa or lymphatic blockage. In these instances again, the endogenous fractional degradative rate may be unchanged or even depressed, but the total amount of albumin lost from the body per day may be increased. The liver, the site of normal albumin production, does not degrade significant amounts of albumin. However, the hepatocyte has a specific affinity for desialylated glycoproteins and degradation of ceruloplasmin may be regulated by this mechanism (22). However, if albumin were to penetrate into the liver cells, the capability for albumin degradation is certainly present via lysosomal enzymes. The degradative system handles new and old albumin molecules alike and this system is probably saturated with albumin molecules at all times. It is only when more sites become available for albumin degradation that the fractional rate would increase.

Much more information is available when we discuss albumin synthesis. The albumin molecule is synthesized in the liver by a polysome bound to the microtubular structure, endoplasmic reticulum. It is transported rapidly from this microtubular system and the smooth endoplasmic reticulum to the Golgi apparatus (28), and from these extruded in some fashion directly into hepatic plasma (6). Potassium concentration plays an important role in the rate of release of preformed albumin and it has been postulated in addition that the albumin molecule may actually be formed as a precursor molecule awaiting discharge from an appropriate stimulus (29). The normal liver synthesizes albumin at about ⅓ capacity, and electron microscopic studies have found albumin to be intracellular in about ⅓ of the hepatic cells (30). This finding should however not be taken as conclusive proof that not all liver cells have the potential for the synthesis of serum albumin. Once synthesized, albumin is deposited into hepatic plasma directly, mainly bypassing the hepatic lymph, and drainage of the thoracic duct has not impeded the appearance of newly synthesized albumin into the plasma.

The most important factor controlling albumin synthesis is probably the level of nutrition and more directly the concentration of amino acids at the site of albumin production (31). A fast for as little as one-half a day, decreases albumin synthesis by one-half and results in a loss of hepatic RNA and disaggregation of the membrane-bound polysome (32, 33). In this state, the hepatic polysome is extremely sensitive to small concentrations of tryptophan, and the addition of tryptophan to the liver obtained from a fasted animal results in rates of albumin synthesis significantly exceeding the rate found in fed control preparations. This finding fits with the fact that the m-RNA for albumin is relatively stable and long-lived and, thus, when reaggregation is stimulated in some fashion by tryptophan, the primary m-RNA available for this reaggregated sytem is coded for serum albumin (33, 34). Excess tryptophan administered in the perfusate to a liver derived from a fed donor had no effect.

It was these observations which stimulated studies on the influence of the prior nutritional state on the protein synthetic response to hepatic toxins such as $CCl_4$ and alcohol. Both these agents result in rapid alteration of the hepatic subcellular system for the synthesis of protein (35, 36). Attachment of the RNA to the endoplasmic reticulum is altered and the ribosome is dispersed into ribosomal subunits. These changes should result in rapid loss of albumin synthesizing capacity. Both alcohol and $CCl_4$ have been shown to cause between 70–90 percent decreases in rates of albumin production (35, 36). Within hours following exposure to alcohol, the liver from a fed donor can be made to recover its albumin synthesizing capacity if alcohol is removed from the system and the liver given a chance to reform its polysomal system prior to measurement of albumin synthesis (37). $CCl_4$ toxicity, however, is not characterized by an equivalent degree of recovery. Excess tryptophan in the alcohol studies were found not only to protect but to partially reverse the toxic effects of the alcohol and albumin production rose rapidly to approach to about 70–90 percent of the control levels even though alcohol was still present in the perfusate at levels of 220 mg percent. The presence of 10 mM tryptophan in the perfusate with a liver derived from an animal that had received $CCl_4$ is not nearly as effective and albumin production increases to only 40–50 percent of the control rate.

Other factors also influence albumin synthesis. Osmotic pressure as we have indicated results in a decreased rate of albumin production and the mediator for this system is located in the interstitial space of the liver (38). This space contains less than one-tenth the concentration of albumin that is found within the plasma and in lymph which are in equilibration with the hepatic interstitial space. The explanation of this fact probably resides in the observation that the matrix of the hepatic interstitial space is in the form of a gel which in itself excludes from solubility other large molecules. Thus, the small amount of albumin located in the available hepatic interstitial space is probably excluded from a large fraction of this pool such that the concentration of albumin within the liver may well approach that in the plasma and lymph surrounding hepatic interstitial spaces (39).

During acclimatization to heat, albumin synthesis is markedly depressed. No such effect was observed in the cold, however. This change may well be related primarily to altered nutrition and in turn to decreased thyroid function, both occurring during hot acclimatization (40).

Optimal albumin synthesis may well require a fine hormonal balance, and thyroid, cortisone, and growth hormone have been shown to be necessary in in vitro situations for albumin production to proceed effectively (11). Hydrocortisone has been shown to stimulate albumin production directly when added to hepatic cells produced in culture (41). Likewise insulin may be important for the optimal synthesis of albumin although diabetes mellitus is not characterized by an altered albumin synthetic rate (42).

Other diseases have been characterized by depressed serum albumin level, particularly cirrhosis of the liver and nephrosis. The low serum albumin level seen in cirrhosis has been considered to reflect the inability of the diseased hepatic cell to synthesize albumin. However, the serum albumin level is only the complex end result of distribution, degradation, and synthesis, and recently albumin synthesis has been observed to be increased or normal in two-thirds of a group of subjects with severe hepatic dysfunction. These patients were studied for one week of hospitalization after they had been removed from heavy alcoholic consumption and placed on adequate nutrition. That these livers are capable of responding normally to excess stress has been shown in a study where cortisone administration resulted in increments in albumin production in patients with cirrhosis (44). These studies, however, do not indicate the rates of albumin synthesis when patients with cirrhosis are still consuming large quantities of alcohol and are also under the influence of a poor nutritional intake. The low serum albumin level in these cases must represent some discrepancy between synthesis and degradation and distribution.

Likewise in nephrosis the liver cell is capable of marked increments in albumin synthesis even though the serum albumin level remains markedly depressed (45). Probably, in this disorder the lowered albumin level again does not represent the maximum capability of the liver due to the fact that altered nutrition may play a role in inhibiting the hepatic response.

The synthesis of albumin is a good example to use to study the adaptation of the liver to stress—whether this be nutritional, hormonal or disease-produced. In vivo regulation is markedly complex, however, and studies at the subcellular level with the isolation of specific effects on the albumin synthesizing machinery (46) are needed and are still in the future. Dr. Berson's superb ideas and his development of basic concepts concerning the measurement of protein metabolism have provided work for many laboratories. His thoughts will stimulate all of us for a long, long time.

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# The Effects of Overload on Cardiac Muscle Protein Synthesis Leading Ultimately to Hypertrophy. How to Approximate the "True" Rates of Protein Synthesis in the Face of Lack of Intracellular Homogeneity

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It was my good fortune to have taken part of my medical and cardiac residency training program at the Bronx Veterans Administration Hospital when Solomon Berson assumed the role of the Chief of the Radioisotope Service. During that year, I was able to participate in a research problem with Dr. Berson and Dr. Rosalyn Yalow and was exposed to their brilliant research thinking for the first time. In the three years that followed my residency, I was appointed as their first postgraduate research associate. During this period, the clarity of Sol's thinking and his brilliance continued to help work out problems which arose both in our combined projects and in the individual research problem of cardiac potassium transport which I initially entered. During my years as his "Fellow", he always found time to discuss my problems and never spoke or acted harshly towards his associates. The stimulation and attitude of good feeling in that laboratory is one I shall never forget.

The discussion presented below concerns the development of some of the ideas about cardiac potassium cellular movement, the finding that intracellular cardiac potassium may be compartmentalized, and how this background influenced thinking in cardiac protein synthesis, in that measurements of this synthesis using labeled amino acids may be influenced by compartmentalization of the amino acid used.

The early studies examining intracellular cardiac $K^+$ movement involved the use of radioactive $^{42}K^+$, which was injected into frogs 24 hours prior to sacrifice (1). The hearts were then removed and placed into a perfusion chamber in which they were perfused with nonradioactive Ringer's bicarbonate buffer and electrically stimulated to maintain the cardiac rhythm. The hearts within the perfusion chamber were bathed with rapidly changing bath solution so that any radioactivity released by the heart by perfusion was rapidly removed from the chamber. The chamber was placed into the well of a well-type scintillation counter, and, in essence, the counter recorded the $^{42}K^+$ activity remaining in the heart during perfusion. In this way, the rate of washout of the $^{42}K^+$ could be ascertained. Since $K^+$ contents were the same before and at the end of perfusion, the heart was in a steady state with respect to $K^+$ and influx was equal to efflux. It soon became evident that potassium within the heart did not

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exchange or move out as a single rate but as at least two distinct rates, with half of the $K^+$ leaving at a rate four times as rapidly as the remainder. The faster moving $K^+$ was not extracellular $K^+$ since the latter represented only a very small fraction of the total, while the rapidly exchanging component consisted of at least 50 percent of the total cardiac $K^+$. Furthermore, by varying the time of equilibration of $^{42}K^+$ in vivo prior to sacrifice, we were able to show that the size of the rapidly exchanging compartment also varied.

Of more importance, were the buildup and washout experiments carried out in the same heart (1). In this latter preparation, the nonradioactive working heart was perfused with Ringer's solution containing $^{42}K^+$ and the buildup of radioactivity recorded. When maximum radioactivity had been reached, the same heart was then perfused with nonradioactive perfusate and a washout of $^{42}K^+$ ascertained. Both the buildup (equilibration study) and the washout (efflux study) showed that $K^+$ within the cell moved, at least physiologically, as if there were two (or more) compartments (1). Similar findings were obtained with the guinea pig heart in vitro (2). Alterations of temperature and digitalis glycosides such as ouabain were found to profoundly affect the exchange of only one of the compartments (2, 3).

At this writing, it now appears rather strange that acceptance of intracellular compartmentalization met with such initial resistance in models involving skeletal muscle and nerve tissue, which were not entirely comparable to a working and perfused heart. One of the reasons for the resistance was the possiblity that diffusion delay through the extracellular space caused a two-compartment system with $K^+$. However, measurements of diffusion through the extracellular space in the perfused beating heart supported earlier data that $K^+$ in the rapidly exchanging fraction was not extracellular $K^+$ since the washout of extracellular ions as $^{24}Na^+$ and $^{131}I^-$ was at a rate 25 times greater than that of the rapidly exchanging intracellular $K^+$ compartment (4). Despite the above, for nearly ten years intracellular cardiac potassium was assumed to exchange as a single totally exchangeable pool, but then newer data began to appear confirming the original findings that the kinetics of $K^+$ exchange in the cardiac muscle cell could not be explained unless there was more than one physiological compartment within the cell (5, 6).

The significance of compartmentalization is still unclear, but recent morphologic studies of cellular structure, particularly in muscle, indicate such complexity of intracellular arrangement that, on morphologic grounds alone, it would be unwise to assume that the intracellular pool of any ion or small molecule behaves as a single unified pool at all times.

Early in the 1960's we became interested in cardiac protein synthesis particularly in stress and again encountered the problem of physiological compartmentalization. In earlier studies, the measurement of "protein synthesis" usually involved a single injection of a labeled amino acid into the experimental animal and the measurement of the incorporation of this amino acid into the protein of the organ to be tested. The methods used were certainly of value in a qualitative sense, and studies with labeled methionine and glycine indicated that there was an increased incorporation of the labeled amino acid into protein, beginning two days following the operation for experimental aortic stenosis (7, 8). However, the quantitative aspects of the problem were open to question. In view of the fact that there was a single injection of the labeled amino acid, the tracer would have a constantly changing specific activity in the extracellular space, hence a changing activity within the intracellular pool. Since the latter was not measured, the early values for protein synthesis were approximations only.

In order to minimize this problem of changing isotopic labeled amino acid levels, cardiac protein synthesis was studied in vitro in the young guinea pig heart where hearts were perfused with a constant concentration or activity of $^{14}$C-labeled lysine and for one, two, or three hours at control and elevated aortic pressures with amino acid fortified oxygenated Krebs-Henseleit solution. "Protein synthesis" was calculated as $\mu$moles lysine incorporated into cardiac protein/g protein N from the formula:

$$\frac{\mu\text{Ci-lysine/g protein N}}{\text{14C lysine specific activity in perfusate } (\mu\text{Ci}/\mu\text{mole})}$$

The perfused hearts maintained contractility for five hours without decrease in amplitude, and failing hearts were not included. The average protein synthetic rate in control left ventricles was 5 to 6 $\mu$moles lysine incorporated into 1 g protein N/hour or 18 $\mu$moles in three hours. In contrast, elevation of aortic pressure resulted in a 70 to 80 percent increase in protein synthesis in the left ventricle after three hours of such stress. These stressed preparations were called "overloaded" (9).

It was apparent in the earlier studies that the values for protein synthesis were minimal values since the specific activity of the $^{14}$C-lysine in the intracellular pool was found to be lower than that of the perfusate although it reached a plateau after the first 30 minutes of perfusion. Of importance was the finding that this intracellular $^{14}$C-lysine specific activity was the same in control hearts and in those subjected to aortic hypertension (9, 10). Thus, any increased incorporation of the label in overloaded hearts was not due to the artifact of a different intracellular $^{14}$C-lysine pool or transport.

Our investigations now proceeded to test both the quantitative aspects of cardiac protein synthesis and the factors which initiate the increased left ventricular protein synthesis in overload.

One of the ways to avoid the problems of variations of intracellular distribution of the labeled amino acid in different pools was the evaluation of subcellular systems after the left ventricle was subjected to stress. Protein synthesis by microsomes isolated from overloaded guinea pig left ventricles was studied in a cell-free system. The microsomes containing sarcoplasmic reticulum and polysomes were incubated with $^{14}$C-leucine and the incorporation of the labeled amino acid into microsomal protein measured. As early as one hour after onset of elevated aortic pressure, the microsomes taken from the overloaded left ventricles showed increased incorporation of the labeled amino acids into microsomal protein. Similar findings were seen with labeled lysine and phenylalanine (11).

The increased protein synthesis by microsomes isolated from overloaded hearts was inhibited by perfusion with actinomycin-D. The latter did not affect control microsomes. Since actinomycin inhibits RNA synthesis, the data directed attention to the possibility that one of the earlier changes leading to increased protein synthesis in overloaded hearts was an increased synthesis of RNA.

With studies using $^3$H-uridine, the type of RNA which was affected was found to be the group of heavier aggregates of polysomes from overloaded left ventricles, since this group contained the most active $^3$H-uridine activity. The latter was prevented by actinomycin in the perfusate (11). Further evidence that the RNA fraction initially stimulated may be m-RNA was obtained in studies of RNA polymerase activity in nuclei obtained from control and overloaded left ventricles perfused for 10 min. to 3 hrs. (12)

It was found that nuclear RNA polymerase activity in $Mn^{2+}$ [favoring formation of a DNA-like RNA or m-RNA (13)] was increased by 20 minutes of overload stress followed by a later increase in activity of $Mg^{2+}$ stimulated type (or ribosome-like RNA). Tracing changes back even earlier led to the finding that adenylcyclase activity was increased in overloaded left ventricles as early as 10 minutes after onset of stress (14). In light of the evidence that adenylcyclase activity may be related to histone phosphorylation (15) and the latter related to RNA polymerase activity (16), the findings pointed to a train of events in the left ventricle following the onset of aortic hypertension, which included increased adenylcyclase activity, increased nuclear RNA polymerase activity, increased m-RNA synthesis, and then r-RNA synthesis, polysome protein activity and augmented protein synthesis. This sequence of events has been supported by recent data showing increased spermine and spermidine activity early in similar cardiac stress which are directly related to RNA synthesis (17).

Measurements of activities of subcellular particles are not subject to the same problems of amino acid intracellular pool distribution since incubation occurs in known and controlled media. However, studies of the different proteins involved in reaction to stress involved going back to the intact heart and to the problems of the specific activity of the precursor amino acid. It was soon realized that possible physiological "compartmentalization" not only referred to ions as $K^+$ but to other molecules as ATP, 3′5′ cyclic AMP, etc. (14). Hence, the calculation of protein synthesis based on measurements of extracellular or perfusate activity without information of the amino acid precursor pool within the cell were approximations only.

The degree of error also depends on the purity of the isolated protein to be measured, the rate of the equilibration of the circulating labeled amino acid with the intracellular pool, and the equilibration of the precursor pool (t-RNA amino acid) with the general intracellular pool. Furthermore, in nonperfused tissues such as papillary muscle preparations or tissue slices, there are added problems in measuring protein synthesis as the viability of inner slices (18), and the delay of diffusion of the labeled amino acid through the nonperfused layers of tissue (4).

The use of the amino acid lysine to measure cardiac protein synthesis helped open the door to the problem of unequal intracellular amino acid distribution. It had long been known, both in liver and heart, that the intracellular $^{14}$C-lysine specific activity was not the same as that of the perfusate even after several hours of perfusion with a constant concentration of the label (9, 10, 19). It was postulated (19) that in such perfusions, protein breakdown released nonradioactive lysine into a large intracellular pool which, at least in the early hours of perfusion, diluted the intracellular free lysine pool and hence reduced the specific activity of $^{14}$C-lysine which entered from the perfusate. Hence protein synthesis calculated from the formula utilizing the perfusate specific activity mentioned in the earlier section would be incorrect; for example, during 3 hours of perfusion of the heart with $^{14}$C-lysine, the intracellular $^{14}$C-lysine specific activity was 0.6 that of the perfusate at 30 min and 0.7 that of the perfusate at 180 min. Therefore, calculations of protein synthesis would more closely approximate the true value if they were based on the mean intracellular specific activity.

The problem of intracellular amino acid pools was further complicated by attempts to measure protein degradation. Usually, the methods involved injecting a labeled amino acid into the intact animal, a period of equilibration of several days to permit labeling of protein, and finally estimating protein degradation by measuring the rate of disappearance of the label in the protein of the organ studied. It was readily apparent that, depending on the amino acid used, the rates of degradation varied greatly in identical organs and species (20). From the information obtained with $^{14}$C-lysine, it was also evident that degradation of previously labeled proteins released $^{14}$C-amino acids into some intracellular pool or pools from which they were reutilized into new protein synthesis; the greater the reutilization of the amino acid, the slower the apparent rate of degradation. In part, we were able to overcome the reutilization of amino acids released by protein degradation by inhibiting protein synthesis with puromycin (21). At appropriate levels, puromycin inhibited 98 percent of protein synthesis and did not affect contractility in vitro. Under these conditions the washout of free released $^{14}$C-lysine doubled indicating that the apparent rate of degradation measured with $^{14}$C-lysine in previously labeled protein was too slow by 50 percent due to reutilization of the amino acid.

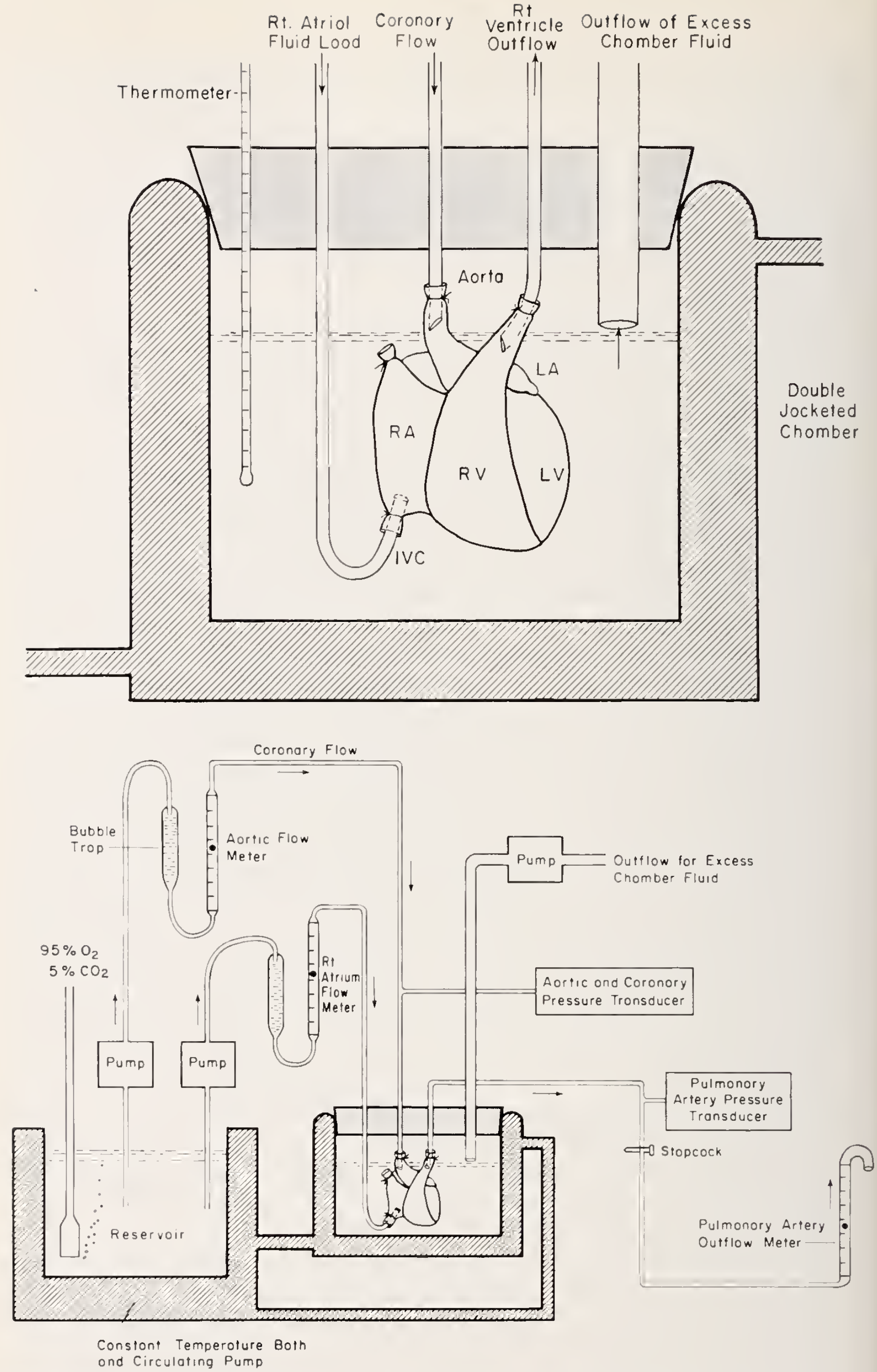
Calculations of cardiac protein synthesis based on earlier data measuring $^{14}$C-lysine incorporated into protein from a mixed intracellular pool in the perfused baby guinea pig heart gave values of 0.17 $\mu$moles of lysine incorporated into the average heart protein each hour. In contrast, the amount of $^{14}$C-lysine released by protein degradation and available for new protein synthesis calculated from the degradation data was double the above figure. This discrepancy led us to examine the protein precursor pool of lysine. According to present theory, this is the t-RNA-lysine pool available for direct protein synthesis. After perfusion with $^{14}$C-lysine for three hours, the RNA-bound $^{14}$C-lysine-specific activity was approximately one-half that of the mixed pool. If incorporation of lysine were now calculated from the $^{14}$C-lysine specific activity of the t-RNA pool then the synthesis figures would more closely approximate the estimates obtained from degradation data. Thus, evidence in the perfused heart indicated that the precursor pool for lysine may not be identical to the mixed intracellular pool—hence, compartmentalization at least in a physiological sense.

Here again, as with the $K^+$ story, initial resistance to this thesis was strong but evidence is now becoming available that some amino acids may behave in an intracellular compartmentalized way. Thus, Rhigetti et al have presented data that phenylalanine and leucine t-RNA pools may be different from the mixed intracellular pool in the liver (22).

Earlier studies in vivo and in tissue slices indicated that there was a proliferation of connective tissues after induced aortic stricture (23, 24). Awareness of the intracellular pools has allowed more accurate measurements of specific protein synthesis during the periods very early in stress. Thus, in vitro, myosin synthesis was increased after three hours of aortic hypertension while myoglobin and collagen synthesis remained unchanged during the early periods (10).

A major problem in the study of hypertrophy has been the differences in the types of hypertrophy which occur following increased aortic pressure or pressure overload versus aortic or mitral insufficiency or flow overload. The in vivo or in vitro models could not be prepared without the increased aortic pressure or increased left ventricular flow also altering coronary flow or pressure. Since alterations in coronary flow per se also could effect changes in protein synthesis (9), it was of importance to attempt to separate the stress imposed on ventricular musculature from the circulation supplying this muscle. A recent approach in our laboratory has allowed an attack on this problem, and some preliminary data are presented below. The young guinea pig heart is prepared for perfusion in vitro by cannulating the inferior vena cava, ligating the superior vena cava, and cannulating the pulmonary artery. Perfusion is directed into the right atrium, and right ventricular pressure is controlled by a microvalve connected to the pulmonary artery cannula which is also in line with a pressure transducer. The flows are monitored by flow meters connected in line. Coronary flow and pressure are maintained by cannulating the proximal aorta above the aortic valve making sure to maintain the integrity of the valve. A flow meter and pressure transducer in this separate perfusion line allows measurement both of coronary flow and of aortic and coronary artery pressure (Figs. 1 and 2).

With this new perfusion method, we have been able to alter right ventricular work due to increased pulmonary artery resistance without changing coronary perfusion. Similarly, it has been possible to alter right ventricular perfusion flow without changing coronary flow. In evaluating the data, it should be noted that the baby guinea pig heart with average cardiac weight of 0.9 grams is a low pressure system (21). The preliminary data demonstrate that when the coronary artery flow is maintained at a constant level, increased right ventricu-


Rt. Atrial Fluid Load
Coronary Flow
Rt Ventricle Outflow
Outflow of Excess Chamber Fluid
Thermometer
Aorta
LA
RA
RV
LV
IVC
Double Jacketed Chamber
Coronary Flow
Bubble Trap
Aortic Flow Meter
Pump
Outflow for Excess Chamber Fluid
95% $O_2$
5% $CO_2$
Rt Atrium Flow Meter
Aortic and Coronary Pressure Transducer
Pump
Pump
Pulmonary Artery Pressure Transducer
Stopcock
Reservoir
Pulmonary Artery Outflow Meter
Constant Temperature Bath and Circulating Pump

Figs. 1 and 2. Apparatus for measuring myocardial protein synthesis in the face of altered pressure or volume load in the right ventricle while maintaining constant coronary perfusion.

TABLE I

*Correlation between Right Ventricular Pressure and Fluid Load and Protein Synthesis after Three Hours of In Vitro Perfusion**

| Right Ventricular Peak Systolic Pressure (mean mm Hg) | Right Ventricular Protein Synthesis** |
|---|---|
| 7.8 | 37.9 |
| 10.0*** | 46.3*** |
| 13.6 | 59.8 |
| 16.5 | 61.1 |
| **Right Ventricular Perfusion Load (mean ml/min)** | |
| 1.8 | 61.1 |
| 1.9 | 55.0 |
| 2.1 | 37.4 |
| 2.6 | 37.4 |
| 3.6 | 37.9 |

* Coronary flow was constant in all of the studies (3.8–3.9 ml/min).

** $^{14}$C-lysine incorporation into protein expressed as μmoles incorporated/g protein N calculated from the $^{14}$C-lysine incorporated into protein and the intracellular $^{14}$C-lysine specific activity.

*** Mean of two separate studies.

lar work due to elevated pulmonary artery pressure results in increased protein synthesis while no such increase may be seen even though the fluid load is doubled (Table I). The data do not imply that protein synthesis will not increase with flow overload, but do indicate that at least within three hours in vitro, increased pressure produces a response much more rapidly than does increased flow per se.

It has been a long road since compartmentalization of potassium in heart muscle was first suspected, but it has been my hope to be able to extend the clarity of Sol Berson's approach to research problems. I feel with deep conviction that the attitudes, excitements, and pleasures first experienced while working in Sol's laboratory will remain with me always.

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# Possible Clues in the Continuing Search for the Subcellular Basis of Thyroid Hormone Action

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Fully 90 years have passed since the hormonal function of the thyroid gland has been recognized (1, 2) and over 80 years since the introduction of thyroid replacement in the treatment of hypothyroidism (3). These early achievements served as a potent stimulus for research, and considerable progress has been made in elucidating many aspects of thyroid hormone physiology and biochemistry. The principal thyroactive substances, L-thyroxine ($T_4$) and L-triiodothyronine ($T_3$), have been identified and synthesized. Major steps in intrathyroidal hormonal synthesis have been clarified and substantial advances have been made in describing the peripheral transport, distribution and tissue metabolism of $T_4$ and $T_3$. One of Dr. Berson's outstanding achievements was his analysis of the quantitative aspects of thyroid hormone economy with special reference to the pathways of iodine metabolism (4).

Despite this long-standing familiarity (or overfamiliarity) with the thyroid hormones, the molecular and cellular basis of hormonal action remains a tantalizing riddle. Many of the biochemical consequences of thyroid excess and thyroid hormone deficiency are known, but there is no general agreement about the sequence of events which characterizes hormonal action within the cell. In fact, there is considerable controversy about the subcellular site of the initiation of these events. Thus, a number of investigators (5–8) have suggested that thyroid hormones interact directly with the mitochondria, whereas Tata and associates (9, 10) have placed primary emphasis on early intranuclear events, suggesting that later biochemical findings represent the effects of transcriptional processes. These and other problems relating to current theories about hormonal action have been extensively reviewed by Wolff and Wolff (11). In the following essay I should like to summarize recent data from our laboratory which may provide some insight into these problems. Specifically, I should like to comment on the following aspects: (1) The significance of the in vitro interaction between thyroxine and mitochondria; (2) The concept of a long-lived hormonal messenger; and (3) The recent demonstration of specific nuclear binding sites for $T_3$. My principal collaborators in these studies have been Dr. Martin I. Surks, Dr. Harold Schwartz, Dr. Diona Koerner, and Dr. Armirav Gordon who was on leave of absence from the Hebrew University, Hadassah Medical School, Jerusalem.

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### 1. *Differences between the in vivo and in vitro stimulation of mitochondrial function*

Buchanan and Tapley (6) have recently reported that $T_4$ added in vitro to a simple suspension of rat liver mitochondria stimulates the incorporation of amino acids into mitochondrial protein. Since a similar stimulation of mitochondrial function has also been observed after the in vivo injection of thyroid hormones into the rat (12, 13), Buchanan and Tapley have proposed that the in vitro phenomenon can serve as a useful model for studying the biochemical basis of thyroid hormone action. Recent studies, however, carried out by Gordon in our laboratory (14) have raised what appear to be serious questions about the applicability of the proposed in vitro model.

A major difference between the in vitro and the in vivo systems is that albumin added to the in vitro incubation mixture reverses the enhanced rate of amino acid incorporation into mitochondrial protein (Fig. 1) whereas this is not the case following the addition of albumin to mitochondria stimulated after in vivo injections of $T_4$ (Fig. 2). In vitro reduction in $T_4$ stimulation appears to be proportional to the amount of bound $T_4$ removed from the mitochondria by albumin as determined by residual mitochondrial counting rates of labeled $T_4$. Furthermore, when isotopically labeled $T_4$ is injected along with nonradioactive $T_4$ the small residual radioactivity in the mitochondria, which may or may not be $T_4$ chemically, could not be extracted with the albumin. In essence, these

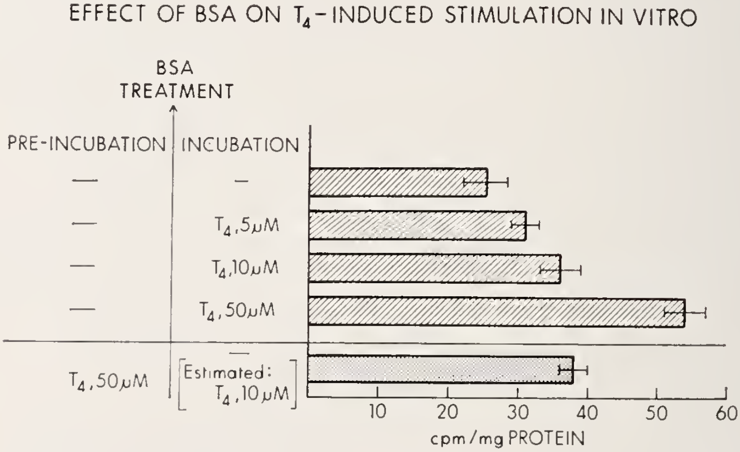

FIG. 1. Mitochondria were preincubated at 37°C for seven minutes in the presence and the absence of $T_4$. Bovine serum albumin was then added to all incubation flasks, which were then immediately centrifuged and washed at 0–2°C. Next, the mitochondria were incubated with $^{14}$C-leucine at 37°C for 20 minutes in the presence and in the absence of $T_4$ as indicated. In other experiments with variable periods of incubation with $T_4$ it was shown that a seven-minute exposure of $T_4$ results in maximal stimulation of amino acid incorporation into mitochondrial protein. The results of the experiment illustrated show that stimulation of mitochondrial protein incorporation after preincubation with 50 μM $T_4$ followed by albumin treatment corresponds to the expected stimulation with 10 μM $T_4$. From radioactive $T_4$ counts, albumin was effective in extracting only 80 percent of $T_4$ from the mitochondria. Thus, in vitro stimulation appears to be related to exchangeable $T_4$ bound to the mitochondria (14). ± SEM indicated.

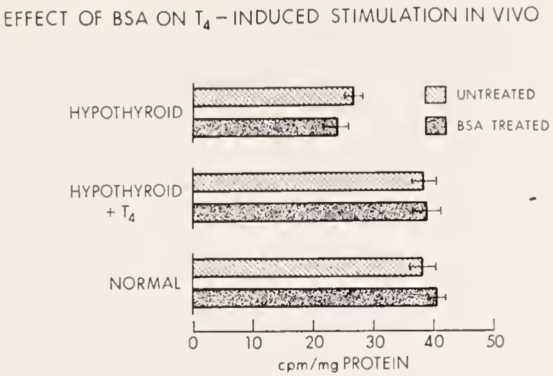

FIG. 2. Hypothyroid animals were pretreated with 100 μg $T_4$ intraperitoneally for three days. Rats were killed 84 hours after the last injection of $T_4$. The albumin was unable to reverse the $T_4$-induced increase in amino acid incorporation into mitochondrial protein. Moreover, albumin failed to exert any significant effect on amino acid incorporation into mitochondria from hypothyroid or intact animals (14). Incubation was carried out with the same procedure used in the experiments illustrated in Figure 1.

results indicate that the in vitro effect appears to be at least roughly proportional to the exchangeable $T_4$ bound to the mitochondria whereas this appears not to be the case under in vivo conditions.

It is widely recognized that in most in vitro models the required concentration of $T_4$ is far in excess of the concentrations of hormone observed under physiologic conditions in the intact animal (11). A similar discrepancy was observed in the case of the $T_4$-induced increase in amino acid incorporation into mitochondrial protein. Estimates based on the counting rates of labeled $T_4$ indicated that at least a 1000-fold excess of $T_4$ was required in the in vitro model in order to produce a similar degree of mitochondrial stimulation observed after the in vivo injection of hormone (Fig. 3).

Apparently supraphysiologic hormone concentrations for in vitro models have been justified by the following argument. In order to deliver a critical concentration of $T_4$ to highly specific subcellular sites a very high concentration of ambient $T_4$ is required. In the intact animal, however, different and more physiologic pathways are available for the delivery of hormone to the same subcellular sites. Thus, high concentrations of thyroid hormones in the ambient fluids are not required in the intact animal. This argument, however, cannot be applied to mitochondrial amino acid stimulation since one would have anticipated that albumin should be able to remove $T_4$ from the suspected mitochondrial sites both after in vivo injection and after in vitro addition. Failure to observe reduction in hormonal effect after $T_4$ injection thus is inconsistent with the concept that similar mechanisms are responsible for the stimulation of

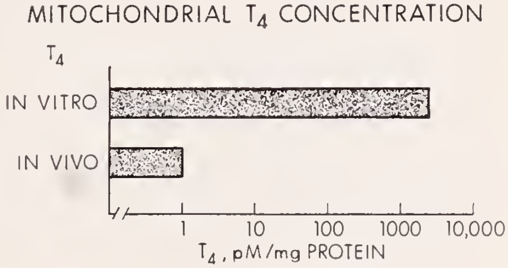

FIG. 3. Indicated are the estimated $T_4$ concentrations required to produce a 26 percent increase in amino acid incorporation into mitochondrial protein, after in vitro addition and after injection of $T_4$ into the intact animals (14).

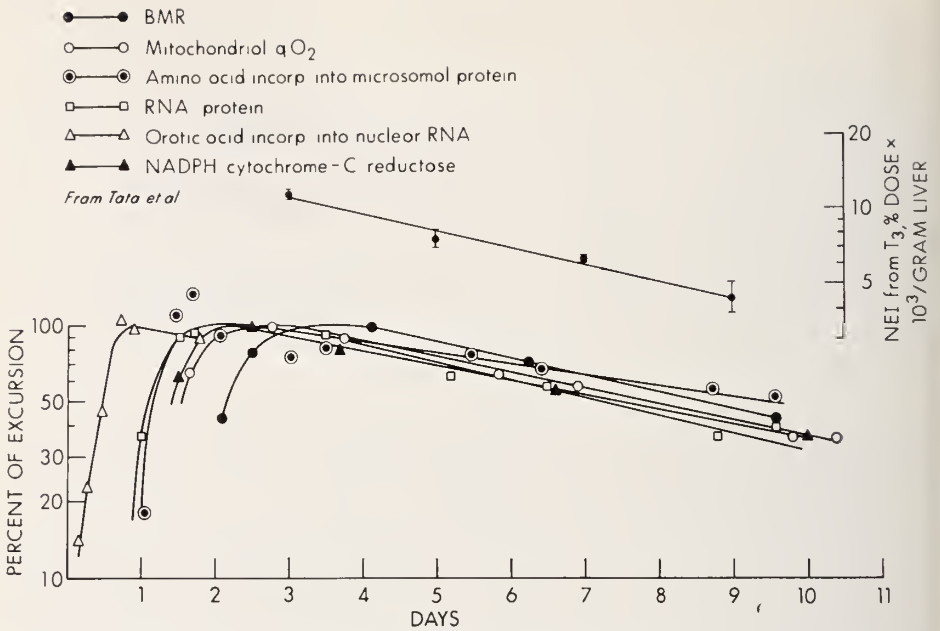

Fig. 4. The sequential appearance and decay of tissue effects in the liver following the injection of 20 to 30 μg of $T_3$ in thyroidectomized rats are plotted on semi-logarithmic coordinates. The primary data is taken from Tata and associates (16–18). Note that no hepatic function is maximal prior to 19 hours and that all functions appear to decay with a common t ½ of four to six days. The t ½ of exchangeable $T_3$ in this preparation varies from between four and six hours. Note the similarity in the fractional decay of the tissue functions and the decay of nonextractable hepatic radioactivity following injection of 30 μg $T_3$ labeled with $^{125}$I-$T_3$ (15).

mitochondria under in vitro and under in vivo conditions. It would be of interest to apply similar criteria to other proposed in vitro models of hormone action.

### 2. *Evidence favoring the existence of a long-lived thyroid hormone "messenger"*

A distinctive aspect of thyroid hormonal action in comparison with most other hormones is the marked inertial property which characterizes the onset and offset of tissue effects. Thus, following the injection of a hormone pulse, a "lag phase" is observed before hormonal action is detectable. Less often stressed is the perpetuation of tissue effects after the hormone has been metabolized. This is illustrated in the composite graph (Fig. 4) (15) prepared from the primary data obtained by Tata and coworkers (16–18). In these experiments a single intraperitoneal injection of 20 to 30 μg of $T_4$ was given to thyroidectomized rats and the following determinations performed serially: orotic acid incorporation into nuclear RNA; RNA:protein ratio; amino acid incorporation into microsomal protein; NADPH-cytochrome C reductase; mitochondrial q02; and total body BMR. The following points will be apparent from the inspection of Figure 4:

a. All of the observed tissue effects of hormone appear to decline with similar t ½'s, in the order of four to six days. In contrast, the t ½ of exchangeable tissue $T_3$ in the rat varies between four and six hours. Thus, the tissue effect of hormone appears to persist long after essentially all of the total $T_3$ has disappeared, an effect which could be termed the "Cheshire Cat phenomenon." *

b. The earliest observed peak effect of thyroid hormone, the rate of orotic acid incorporation into nuclear RNA, occurred 19 hours after the injection of $T_3$. Tata has suggested that alterations in nuclear uptake of nucleotides might precede synthesis of nuclear RNA and that the subsequent biochemical changes in the liver cells are a consequence of these early events.

The finding of what appears to be a common terminal slope for all of the hepatic parameters responses analyzed suggests the existence of a long-lived, rate-determining intermediate. The slow fractional removal of the postulated intermediate (t ½ = 4.5 days) would determine the fractional disappearance of all of the subsequent biochemical steps in the reaction chain. Moreover, if one assumes that the postulated long-lived intermediate messenger precedes all of the measurable hormonal tissue effects, it follows that no hormonal effect can be maximal before the concentration of the presumed intermediate is maximal. On the basis of mathematical considerations developed elsewhere (15) it can be shown that if the t ½ of $T_3$ is four hours, the t ½ of the postulated intermediate is 4.5 days, then the maximal concentration of the intermediate cannot occur before 20 hours. Thus, it should be possible for the peak effect of a given hormonal action to be maximal at any time after 20 hours, but not before 20 hours. This prediction is compatible with the timing of the various peaks observed in Figure 4, given the inherent experimental error in these studies. Moreover, the finding that the maximal rate of the incorporation of orotic acid into nuclear RNA occurs at 19 hours (probably not significantly different from 20 hours) strengthens Tata's conclusion that changes in nuclear metabolism constitute one of the earliest steps in the chain of events which characterize thyroid hormone action in the hepatic cells. A long-lived intermediate thus accounts not only for the observed slow dissipation in hormonal tissue effects but also for the delay in achieving maximal cellular response following pulse injections of $T_3$.

The chemical nature of the postulated intermediate remains an enigma. In this connection it may be of interest that both $T_3$ and $T_4$ are capable of forming what appear to be covalent complexes with plasma proteins. It has been established that following injections of $^{125}I\text{-}T_3$ and $^{125}I\text{-}T_4$ a small but significant proportion of injected radioactivity remains tightly bound to tissue proteins, resisting, exhaustive extractions with ethanol (nonextractable iodine = NEI) (20, 21, 22, 23). Studies with 14-C-ring labeled $T_4$ as well as chromatography of

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* From Lewis Carroll in *Alice's Adventures in Wonderland,* Chapter 6, "Pig and Pepper," The Macmillan Company, New York 1966, p. 62 (19). "'All right,' said the Cat; and this time it vanished quite slowly, beginning with the end of the tail, and ending with the grin, which remained some time after the rest of it had gone. 'Well! I've often seen a cat without a grin,' thought Alice; 'but a grin without a cat! It's the most curious thing I ever saw in all my life!'"

hydrolysates of $T_4$ in plasma NEI indicated that the entire iodothyronine skeleton is incorporated into the complex (22, 23).

The relatively slow fractional turnover of NEI may be pertinent to the problems under discussion (15). Thus, the terminal t ½ in liver derived from the injection of $T_3$ is 4.5 days, a value which corresponds closely to the t ½ of decay in the liver tissue effects illustrated in Figure 4. We have speculated on the possibility that the postulated long-lived intermediate could represent a species of NEI, specifically, a complex between a protein receptor and a hormone or its derivative. It should be emphasized, that this proposal remains far from proved. NEI is distributed to all of the subcellular fractions including microsomal membranes which, presumably, are not involved in hormonal action (24). If this suggestion is correct, one must also assume that the specific NEI which is formed shares the characteristic decay constant of total tissue NEI determined by ethanol extraction. Moreover, the capacity of $T_3$ and $T_4$ to form complexes with proteins may represent a relatively nonspecific chemical property of all the iodothyronines. $T_3$ and $T_4$ can form complexes with liver microsomes (25) and with plasma proteins (26) under what appear to be extremely mild in vitro conditions. Regardless of these considerations, the property of iodothyronines to form irreversible complexes with proteins raises the possibility that thyroid hormones may interact with a specific receptor site to form the postulated long-lived intermediate. Additional studies should help to support or reject this hypothesis.

### 3. *Stereospecific binding sites for triiodothyronines*

In any effort to describe hormonal action at a cellular level it is useful to be able to specify the subcellular site at which the chain of biochemical events is initiated. Recent studies in our laboratory suggest that this site of initiation is situated within the nucleus. Our observations strengthen Tata's earlier suggestions about the primacy of nuclear events in thyroid hormone action.

The observation which led to the identification of specific nuclear $T_3$ binding sites was the finding that the partition of radioactive $T_3$ between pituitary and plasma three hours after injection was closely related to the dose of $T_3$ injected (27). It was shown that the higher the dose injected, the smaller the pituitary: plasma concentration ratio (Fig. 5). This relationship differed sharply from the behavior of other tissue such as kidney, liver, and brain in which the tissue: plasma concentration ratio appears to be independent of the dose injected. Furthermore, the $T_4$ tissue: plasma ratios of pituitary and other tissues are also largely independent of the dose injected. Calculations of the total concentrations of $T_3$ in pituitary based on the estimated $T_3$ concentration in plasma and the specific activity of the injected $T_3$ suggested the existence of highly specific, limited binding capacity sites for $T_3$ which appeared to be close to saturation at normal plasma levels of $T_3$.

These observations prompted the design of experiments to determine whether specific sites in other tissues might have been overlooked because of a preponderance of nonspecific sites in such tissues. This turned out to be the case. When

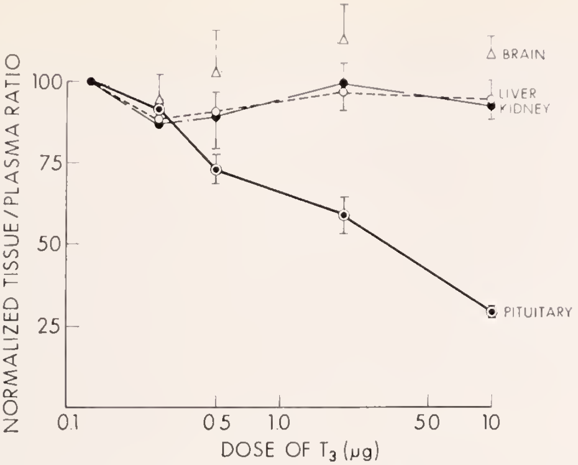

FIG. 5. As the dose of intravenously injected $T_3$ is increased, the pituitary: plasma concentration ratio of $^{125}I$-$T_3$ decreases whereas the tissue:plasma concentration ratio of other tissues appears to be uninfluenced by the injected dose. These results point to the existence of specific $T_3$ binding sites in the pituitary. All values are normalized to 100 at the lowest dose injected. Each point represents the mean of five animals. Bars represent ± SEM (27).

highly purified nuclei were separated from the liver homogenates one-half to 3 hours after injection of $T_3$, the percent of total liver radioactivity associated with the nuclear pellet was found to be strongly dependent on the dose of nonradioactive $T_3$ injected (28) (Fig. 6). Chromatographic analysis showed that the radioactivity both in the total homogenates and in the nucleus was in the form of $T_3$. Analogous studies showed that when $T_4$ was injected, a much smaller percentage of the total radioactivity was bound to the nucleus and the partition of radioactivity between nucleus and the remaining cell was only minimally dependent on the total dose of $T_4$ injected (Fig. 6). When other subcellular components were similarly studied no specific $T_3$ or $T_4$ binding sites were discernible. Nuclear binding sites for $T_3$ were also found in kidney (28).

In preliminary studies (29) we have also demonstrated specific nuclear binding sites for $T_3$ in heart, brain, testis. The findings of specific $T_3$ nuclear sites in brain and testis was of interest inasmuch as these organs have generally been considered to be unresponsive to thyroid hormone, at least in terms of the expected increase in oxygen consumption (30). The significance of specific sites in these tissues is still not clear. Efforts are currently in progress to compare the number of $T_3$ binding sites per mg DNA in a given tissue with the responsivity of that tissue to hormone.

A recent finding of interest to us has been the demonstration that over 50 percent of the total cellular $T_3$ of the pituitary is bound to the nucleus (29). In tissues such as liver, only 8 to 12 percent of total cellular $T_3$ is recovered in the pellet. It is therefore easy to understand why saturation of these limited sites in liver will not make an appreciable difference in total tissue:plasma ratio as the injected dose of $T_3$ is increased and why the total pituitary:plasma ratio is affected under such circumstances.

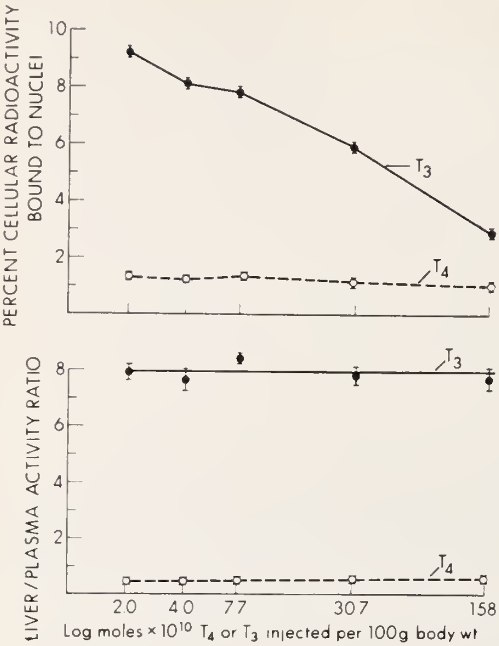

Fig. 6. The percentage of total liver $T_3$ contained in the nuclear pellet is related to the total dose of $T_3$ injected. The binding of $T_4$ to nuclei, however, does not appear to be strongly related to the dose of $T_4$ injected. The total liver:plasma concentration ratios or $T_3$ or $T_4$ also are uninfluenced by the dose. Each point represents a mean of five animals (± SEM indicated). Animals were killed three hours after injections (28).

The identification and the physicochemical nature of the specific intranuclear sites of $T_3$ binding are matters of considerable interest. The interaction between $T_3$ and the nuclear sites appears to be noncovalent since $T_3$ can easily be extracted from the nuclei with ethanol following in vivo injection of hormone. Preliminary experiments after in vivo injection of tracer $T_3$ have shown that nuclear radioactivity cannot easily be removed by the detergent Triton-X. Since this material removes the outer nuclear membrane we have concluded that $T_3$ is bound either to the inner membrane or to another portion of the nucleus.

The receptor site also appears to be heat-labile and subject to destruction by pronase hydrolysis. To date, we have not been able to simulate nuclear binding by addition of $T_3$ in vitro. We have attempted to do this by addition of labeled $T_3$ to total tissue homogenates and by incubating nuclear preparations both with cytosol and with buffer at various temperatures. Such radioactivity as does attach to the nucleus under these in vitro conditions is not displaceable by an excess of nonradioactive $T_3$. Moreover, the radioactive material can easily be removed with Triton-X, suggesting that it is loosely and nonspecifically bound to the outer nuclear membrane (31). In this respect, $T_3$ binding to nuclei appears to differ sharply from previously reported experiments with steroids in which nuclear binding could be replicated under in vitro conditions (32–35). Whether or not cytosol-$T_3$ receptor sites exist which then transport the hormone to the nucleus, in an analogy to the suggested behavior of certain steroid hormones, remains to be determined.

## Conclusion

We hope that the data which have been discussed in this brief review can serve as useful clues in further studies designed to elucidate thyroid hormone action at the cellular level. The demonstration that in vitro stimulation of amino acid incorporation into mitochondrial protein appears to differ significantly from the in vivo phenomenon again emphasizes the caution which must be exercised in the interpretation of in vitro models. The kinetics of onset or hormonal action and the kinetics of dissipation of hormonal effects suggest the existence of a long-lived intermediate messenger, perhaps in the form of receptor-hormone complex. Lastly, the finding of stereospecific nuclear binding sites for $T_3$ in many tissues reinforces previous suggestions that the nucleus is an important early site of hormone action. The much higher avidity of these sites for $T_3$ in comparison to $T_4$ is consonant with other lines of evidence which suggests that the active hormone is $T_3$ rather than $T_4$.

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# Tissue Thyroid Hormone Concentration of Rat and Man Determined by Radioimmunoassay: Biologic Significance

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It has long been recognized that the intensity of hormone action on tissues is, in general, a function of the concentration of the hormone in the circulating blood. In the case of the thyroid hormones, however, two important phenomena have been found to modify the validity of this generalization. The physical state of the hormone in blood (free versus bound fraction) affects the disposal of the hormone, and more recently it has been recognized that conversion of the circulating hormone complex from $T_4$ to $T_3$ plays an important role in the metabolic effectiveness of the thyroid hormone. It is apparent, therefore, that in the evaluation of the action of thyroid hormone on cells the crucial parameter is not the concentration in circulating blood but rather the concentration of thyroid hormones in tissue effective sites.

The validity of this view has been evident for some time but technical problems have made the direct measurement of the concentration of thyroid hormone in tissues extremely difficult. Early attempts to measure tissue hormone levels were unsatisfactory because of inadequacy of methods for measurement of iodine in small quantities. When precise methods for measurement of iodine became available it was possible to measure tissue protein bound iodine (1–3), but there was still considerable uncertainty as to the nature of the products found in tissue, no discrimination being possible among $T_4$, $T_3$ and other putative iodinated protein derivatives.

With the introduction of radioactive thyroxine and triiodothyronine a wealth of data about distribution of labeled hormone in tissues became available but these studies have not given an adequate measure of tissue thyroid hormone levels because there is uncertainty about the size of the intratissue thyroid hormone pool into which the labeled hormone is distributed (or enters after in situ degradation) and the difficulty in defining a steady state for measurement of hormone distribution and metabolism. Perhaps the best attempt to measure tissue thyroid hormone concentration is that of Heninger et al (4) who utilized the method of isotope equilibrium originally introduced by Van Middlesworth (5). This work involves a prolonged period of isotope feeding with $^{125}$I to rats (30 days) to achieve equilibrium followed by chromatographic identification of labeled products in tissue extracts. Such methods are both laborious and clearly unsuited for many kinds of studies in animals and, of course, in humans. Moreover, because of radioactivity decay, uniform labeling of body pools with different rates of thyroid hormone turnover can never be achieved.

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As in the case of so many other fields of endocrinology, the decisive factor which has made it possible to measure the extremely low levels of thyroid hormone in tissue has been the application of the principle of radioimmunoassay. It is a curious fact of historical interest that as early as 1938 it had been recognized that thyroxyl-protein complexes stimulated the formation of serum components which blocked the action of administered thyroxine (6) and by 1963 that immunization of guinea pigs with thyroglobulin led to the formation of gamma globulin antibodies to thyroxine (7) and to a delayed clearance of administered $T_4$ and of $T_3$ from the blood of guinea pigs (8). These observations bear more than a casual resemblance to the contemperaneous observations made by Yalow and Berson in 1960 (9) about the behavior of insulin in diabetic patients. The delay in capitalizing on these observations for developing an immunoassay for thyroid hormones is a reflection of the slow diffusion of new ideas; haptenic immunization with triiodothyronine for production of specific antibodies to $T_3$ was not applied practically for immunoassay until 1970. Since the initial report by Ekins et al (10), a number of workers have reported $T_3$ immunoassays based on immunizations with $T_3$ protein complexes (11–18). More recently, immunoassays for $T_4$ have been developed using thyroglobulin (with its biologically complex iodotyrosyl residues) as the immunizing antigen (19). The major problem in direct plasma immunoassay of iodothyronines has been the high binding affinity of the thyroid hormone to the normally occurring thyroid hormone binding proteins TBPA and TBG. This binding has been circumvented by adding reagents known to dissociate TBG-thyroid hormone-protein complexes such as diphenylhydantoin (15), salicylates (17), tetrachlorothyronine (14), and ANS (8-anilo-1-naphthalene sulfonic acid) (20). The use of a barbital buffer (21) permits the dissociation of thyroid hormone complexes and TBPA.

The increasing activity in this area has been motivated by the recognition of the importance of $T_3$ in thyroid physiology (22–24), the extraordinarily small concentration of $T_3$ which is found in serum, and the current widespread sophistication in the technical aspects of radioimmunoassay.

Our group undertook to develop $T_3$ and $T_4$ immunoassays so that we might examine the concentration of these hormones in blood and tissues under varied physiologic conditions in animals, and pathologic conditions in the human (16, 25, 26). Anti-$T_3$ antibodies were developed in guinea pigs by injecting them with an antigen prepared by complexing $T_3$ to bovine fibrinogen by the glutaraldehyde method of Vance et al (27). Antibodies to $T_4$ were raised in guinea pigs by injection of porcine thyroglobulin following the method of Chopra et al (19). Initial incubation of labeled $T_3$ (or $T_4$) in barbital buffer is carried out with guinea pig anti-$T_3$ (or $T_4$) antibody for one day in the presence of ANS followed by separation of the bound from the free form of the hormone by the coated charcoal method of Gottlieb et al (28). It is important that the plasma added to the standard curve be free of $T_3$ (or $T_4$). This has been accomplished by adsorbing normal plasma with charcoal as recommended by Mitsuma et al (20) and Larson (17). Direct assay of human plasma for both $T_3$ and $T_4$ is readily accomplished in this manner. Alcoholic extracts of plasma or tissues taken to dryness can also be used for assay. In rat plasma, substances interfering with the initial antigen-antibody reaction in the $T_3$ assay are capriciously found in normal plasma and regularly in plasma from hypophysectomized, thyroidectomized and propylthiouracil-treated animals (25). For this reason we now routinely prepare rat plasma samples for immunoassay by extraction by the ethanolic method of Murphy and Pattee (29). An initial plasma volume of 200 $\mu$l is readily concentrated four-fold, thus improving the sensitivity of the assay. The direct $T_4$ immunoassay is apparently not affected by rat plasma which may be a reflection only of the much greater concentration of $T_4$ found in plasma.

Methods for extraction of thyroid hormone from tissue have been adapted from Flock and Bollman (30) and Heninger et al (4). These will be reported in detail (31). The method involves the initial extraction of tissue homogenates with acidified butanol followed by alkaline butanol. More than 70 percent of total tissue radioactivity can be extracted in this fashion but the complete adequacy of this extraction method remains to be fully established in light of the recent findings of Oppenheimer et al about the possible importance of "nonextractable" iodine-protein tissue complexes (32).

By immunoassay we find normal male rat plasma $T_3$ to be 160 pg/ml (high iodine diet) as compared with normal human $T_3$ levels of approximately 100 ng/100 ml. Rat plasma $T_4$ by radioimmunoassay is 5.0 $\mu$g/100 ml and in the human 4-5 $\mu$g/100 ml.

In contrast to the low figures for $T_3$ in rat plasma is the relatively high value in parenchymal tissues (Table I). For example, the concentration of $T_3$ in the kidney is 12 times greater than in plasma, of liver 11 times greater, and in brain 7.8 times greater. Concentrations of $T_4$ in tissue are not very different from

TABLE I

*Tissue and Plasma $T_3$ Levels in Rats Treated either with $T_3$ or $T_4$ for Two Weeks*

| Tissue | Normal | $T_3$-Treated (10 $\mu$g/day) | $T_4$-Treated (25 $\mu$g/day) |
|---|---|---|---|
| Kidney ($\mu$g/100 gm) | 0.192 ± 0.012 | 0.638 ± 0.120 | 0.595 ± 0.089 |
| Liver ($\mu$g/100 gm) | 0.174 ± 0.005 | 0.365 ± 0.060 | 0.376 ± 0.05 |
| Brain ($\mu$g/100 gm) | 0.124 ± 0.006 | 0.160 ± 0.040 | 0.141 ± 0.005 |
| Plasma ($\mu$g/100 ml) | 0.016 ± 0.002 | 0.24 ± 0.02 | 0.100 ± 0.01 |

* Note that kidney and liver $T_3$ levels are nearly equal in the hormone-treated animals, despite the finding that plasma $T_3$ levels are 2.4 times higher in $T_3$ treated animals. This finding suggests that $T_4$ and $T_3$ conversion has occurred in the tissue. In contrast, brain $T_3$ is virtually unchanged by either treatment suggesting a strictly limited uptake for $T_3$ and conversion of $T_4$ to $T_3$ (31). Tissue values not corrected for recovery.

those in plasma. Immunoassay values for $T_3$ in tissue correspond remarkably closely to those reported by Heninger et al (Table II).

This makes it apparent that a concentrating mechanism must exist for maintaining high tissue to plasma gradients of $T_3$. In order to determine whether high intracellular $T_3$ concentrations arise because of selective translocations from plasma or from increased intracellular breakdown of tissue $T_4$ in situ, rats were treated with either $T_3$ or $T_4$ for two weeks (10 μg or 25 μg/day respectively). Amounts were given which raised plasma $T_3$ levels (after $T_3$) to 2.4 ng/ml (15 times normal) and 1.0 ng/ml (6.3 times normal) (Table I). Despite the fact that plasma $T_3$ levels were much higher after $T_3$ than after $T_4$ administration, tissue $T_3$ levels were the same. Thus, tissue:plasma concentration gradients for $T_3$ after $T_3$ injections were 2.85, 1.85 and 0.66 for kidney, liver and brain, respectively and after $T_4$ were 5.95, 3.76 and 1.41. It is clear therefore that tissue conversion, rather than sequestration from plasma, was the more important cause of the high tissue $T_3$ levels.

Further proof that the intracellular degradative process determines the relative concentration of $T_3$ and $T_4$ in tissue comes from the study of the effects of administration of propylthiouracil in rats. This agent, as reviewed by Oppen-

TABLE II

*Values for Tissue Thyroid Hormone as Compared with Radioimmunoassay*

| Tissue | PBI, μg/100 gm or 100 ml | | | Isotope Dilution Rat (4) | | Immunoassay | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Rat (5) | | Human (6, 7) | |
| | Rat (1) | Dog (2) | Human (3) | $T_4$ μg/100 ml or 100 gm | $T_3$ μg/100 ml or 100 gm | $T_4$ μg/100 ml or 100 gm | $T_3$ μg/100 ml or 100 gm | $T_4$ μg/100 ml or 100 gm | $T_3$ μg/100 ml or 100 gm |
| Brain | | | | 0.118<br>(0.08) | 0.097 | 0.833 | 0.120 | | |
| Liver | 25.9 | 5.8 | 8.4 | 1.105<br>(1.12) | 0.197 | 1.300 | 0.152 | 7.7 | 3.20 |
| Kidney | 32.6 | 2.8 | 8.0 | 0.926<br>(1.99) | 0.508 | 0.548 | 0.192 | 2.4 | 0.84 |
| Muscle | 9.5 | 0.6 | 5.8 | 0.117<br>(0.181) | 0.055 | | | | |
| Heart | 21.5 | 1.1 | 6.1 | 0.356<br>(0.610) | 0.151 | | | 1.9 | 0.28 |
| Plasma or Serum | 5.4 | 1.5 | 4.8 | 2.19<br>(4.26) | 0.067 | 5.0 | 0.0142 | 6.8 | 0.110 |

1. Klitgaard et al (1).
2. Carr and Riggs (2).
3. McClendon and Foster (3).
4. Heninger et al (4)—expressed as hormonal iodide. Values for low iodine diet. In parenthesis for high.
5. Nejad et al (31)—expressed as hormone concentration.
6. Sullivan et al (26)—expressed as hormone concentration.
7. Bollinger, Sullivan, and Reichlin, unpublished.

heimer et al (33) and by Nejad et al (31) alters the cellular degradation of thyroxine.

Animals were treated with $T_4$, with $T_3$, and with these hormones individually plus propylthiouracil. After two weeks, plasma and tissue $T_4$ and $T_3$ levels were measured (31) (Table III). Treatment of rats with $T_4$ led to a marked increase in plasma and tissue concentrations of $T_3$, as compared with normal levels. These changes have been interpreted to be due to the peripheral conversion of $T_4$ to $T_3$, as now established by the work of a number of authors (22, 34, 35). When simultaneously treated with PTU, plasma $T_3$ levels of $T_4$ injected rats were reduced to less than six percent of the levels of animals given no PTU. Tissue $T_3$ levels after $T_4$ administration were also markedly reduced by PTU administration. A further suggestion that PTU impaired the conversion of $T_4$ to $T_3$ was the finding that plasma levels of $T_4$ in PTU treated animals were higher than in animals given no PTU. An additional important finding was that tissue levels of $T_3$ after $T_3$ injection were increased by the simultaneous administration of PTU. We conclude from these studies, as did Oppenheimer et al (33) in 1972, that PTU inhibits the deiodination of both $T_4$ and $T_3$.

Work with $T_3$ radioimmunoassay has also given new insight into the role of the thyroid gland in the chronic cold adaptive response. In the rat, exposure to the cold induces both short- and long-term increases in pituitary-thyroid function. Acute release of TSH has been shown to be part of a neurogenic reflex mediated by thermoreceptors in either the skin or pre-optic hypothalamus (36, 37). On the other hand, chronic activation of pituitary-thyroid function as seen in cold adapted animals has not been clearly shown to be due to neurogenic increases in TSH secretion. In fact, the finding that plasma thyroxine (or PBI) are either unchanged or lowered slightly and that fecal thyroxine excretion is increased in cold-adapted rats has been interpreted to mean that pituitary-thy-

TABLE III

*Plasma $T_4$ and $T_3$ Concentration and Tissue (Liver and Kidney) $T_3$ Concentration in Rats Treated with Daily Injections of $T_4$ and $T_3$ and n-propylthiouracil (PTU) for Seven Days*

| Treatment | Plasma | | Tissue $T_3$ | |
|---|---|---|---|---|
| | $T_4$ μg/100 ml ± SE | $T_3$ μg/100 ml ± SE | Liver μg/100 gm ± SE | Kidney μg/100 gm ± SE |
| 25 μg $T_4$ | 10.7 ± 0.8 | .0885 ± .001 | 0.167 ± 0.02 | 0.437 ± 0.047 |
| 25 μg $T_4$ + PTU | 14.9 ± 2.3 | *0.0067 ± 0.001* | *0.027 ± 0.002* | *0.076 ± 0.006* |
| 5 μg $T_3$ | 1.6 ± 0.1 | 0.0197 ± 0.0038 | 0.065 ± 0.006 | 0.141 ± 0.019 |
| 5 μg $T_3$ + PTU | 1.5 ± 0.3 | 0.0138 ± 0.003 | *0.111 ± 0.007* | *0.237 ± 0.027* |

Statistical Comparison: hormone treated vs respective hormone treated + PTU.
Statistical Significance: $p < .001$ is italicized.
All other comparisons not significant statistically ($p > .05$) (Nejad et al (31)).
Values not corrected for recovery.

roid activation in these animals is secondary to an increased rate of peripheral clearance of thyroid hormone (Galton and Nisula (38)).

In view of the importance of $T_4$-$T_3$ conversion in thyroid hormone economy we undertook to study plasma and tissue $T_3$ concentrations in cold adapted rats kept at 4 C for four weeks prior to death. Plasma $T_4$ levels were slightly but significantly less in cold-adapted animals (Table IV) while plasma $T_3$ levels were found to be significantly higher than in controls (16, 24, 39). Most important is the finding that the concentration of $T_3$ in the kidney of cold-exposed animals is significantly greater and that of $T_4$ significantly less than in normals. On the other hand, tissue $T_3$ concentrations of liver and brain are not increased in cold-adapted rats. These observations are interpreted to mean that in the cold-adapted rat there is selective tissue $T_3$ stimulation, not reflected in an increased plasma thyroxine.

This observation adds to understanding of the nature of the pituitary-thyroid response to chronic cold exposure in the rat. Previous studies have shown that there is an increase in plasma TSH and in thyroid function in cold-exposed rats as well as an increase in the activity of hypothalamic TRH synthetase (37). It has been proposed therefore that cold adapted rats have a neurogenic increase in TRH secretion related to cold thermoreceptor reflexes. The increased rate of $T_4$ loss due to gastrointestinal excretion of $T_4$ (related to neurogenic increase in food intake and enhanced enterohepatic circulation and biliary excretion) probably accounts for the fact that the increased secretion of TRH and TSH does not raise plasma $T_4$ levels above the normal baseline "set point." The additional finding that there is a high concentration of $T_3$ in the kidney means that there is also an element of enhanced thyroid hormone stimulation of tissue metabolism which must add an appreciable amount of heat to cold exposed animals to provide "non-shivering" thermogenesis.

Methods of assay of tissue as described above are now being applied in our laboratory to the study of the extrathyroidal tissue metabolism of $T_3$ and $T_4$ in

TABLE IV

*Plasma $T_3$ and $T_4$ and Tissue $T_3$ in Cold-Adapted Rats*

| Tissue | Normal | Cold-Exposed | Statistical significance of difference between normal and cold exposed |
|---|---|---|---|
| | | | *p value* |
| Plasma | | | |
| $T_4$ μg/100 ml | 4.98 ± 0.32 | 4.1 ± 0.24 | $< .05$ |
| $T_3$ μg/100 ml | 0.0127 ± 0.0014 | 0.0229 ± 0.0036 | $< .05$ |
| Brain | | | |
| $T_3$ μg/100 gm | 0.124 ± 0.006 | 0.122 ± 0.005 | n.s. |
| Liver | | | |
| $T_3$ μg/100 gm | 0.176 ± 0.005 | 0.146 ± 0.008 | n.s. |
| Kidney | | | |
| $T_3$ μg/100 gm | 0.192 ± 0.012 | 0.307 ± 0.004 | $< .05$ |

TABLE V

*Tissue and Plasma Levels of Thyroid Hormones in Patients with Chronic Wasting Illness ("Sick Euthyroid")*

| Group | Plasma | Brain | Heart | Liver | Kidney |
|---|---|---|---|---|---|
| Control (patients dying relatively suddenly) | | | | | |
| Free $T_4$ (ng/100 ml) | 1.4 ± 0.2 | | | | |
| $T_4$ ($\mu$g/100 ml) or 100 gm | 7.0 | — | 0.05 | 4.8 | 1.5 |
| $T_3$ ($\mu$g/100 ml) or 100 gm | 0.11 | 0.28 | 0.24 | 0.94 | 0.70 |
| $T_3/T_4$ ratio | 0.16 | — | 0.44 | 0.49 | 0.20 |
| Sick Euthyroid (patients dying after long wasting illness) | | | | | |
| Free $T_4$ (ng/100 ml) | 2.6 ± 1.0 | | | | |
| $T_4$ ($\mu$g/100 ml) or 100 gm | 2.67 | 3.4 | 2.70 | 5.55 | 4.10 |
| $T_3$ ($\mu$g/100 ml) or 100 gm | 0.025 | 0.16 | 0.10 | 0.140 | 0.009 |
| $T_3/T_4$ ratio | 0.09 | 0.12 | 0.05 | 0.03 | 0.05 |
| $T_3/T_4$ Sick | | | | | |
| Ratio of $T_3/T_4$ control | 0.56 | | 0.013 | 0.0061 | 0.25 |

Values not corrected for recovery.

the human (Sullivan et al (26) 1973). It has been found that patients dying of chronic wasting illness have a striking reduction in tissue $T_3$ concentration and a relative increase in tissue $T_4$ levels (Table V). These abnormalities in $T_4$ and $T_3$ metabolism are reflected in the extremely low plasma $T_3$ levels in these patients. The sick euthyroid patient (as pointed out by Sterling and collaborators 40) has a low or normal total plasma $T_4$ and in addition a significantly increased plasma free $T_4$ concentration. These observations have led us to propose that intracellular $T_3$ deficiency in the sick euthyroid patient leads to compensatory increase in TSH secretion: A new set point of plasma-free $T_4$ is the consequence of this change. It is probable that the defect is due to a tissue deiodinase deficiency, but a deficiency of intracellular $T_3$-binding protein is possible. The biologic significance of these changes and its relationship to the event leading up to death have not been determined. That questions of this sort can be raised at all is a tribute to the work of Berson, Yalow, and their collaborators who have given us powerful analytic tools, and the inspiration to use them.

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# Inherited Variations in Thyroxine Transport

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The thyroid hormones, after their release from covalent linkage to the thyroid protein, thyroglobulin, are bound reversibly to three transport proteins in serum: thyroxine-binding globulin (TBG); thyroxine-binding prealbumin (TBPA or PA); and albumin. It has, therefore, been of interest to inquire whether genetically determined variations in these transport proteins exist and, if so, whether they play a role in the physiology of the hormones. The results of such inquiries are summarized in this brief review.

A common phenomenon encountered with most animal proteins is the occurrence of genetic polymorphisms. It is extraordinary that, in the extensive experience with TBG and PA in man since their discovery 20 and 14 years ago, not a single instance of a polymorphism has been detected. The possibility of finding a polymorphism is increased when disparate populations are examined. A study of this kind was carried out with B. Blumberg (1) on 25 sera obtained from different racial groups. Electrophoresis in starch gel, at that time the most useful method for detecting protein variants, was used with $^{131}$I-labeled thyroxine. No polymorphism could be observed in either of these two major transport proteins. However, a polymorphism of the rapid prealbumin band observed previously by Blumberg in the rhesus monkey, Macacus mulatta (2), was shown to involve the thyroxine-binding prealbumin (1). Three variants were observed: a common type having the same mobility as human PA; a rare type migrating just ahead of albumin; and an uncommon type with intermediate mobility. Several years later, electrophoresis in polyacrylamide gel was employed in a study of the mode of inheritance of this polymorphism (3). The fast PA (now designated $P_t$ 1–1) and the slow PA ($P_t$ 2–2) were monodisperse; but the intermediate PA ($P_t$ 1–2) formed five separate bands, two of them identical with $P_t$ 1–1 and $P_t$ 2–2, and all exhibiting thyroxine binding. This led to the conclusion that PA is a tetramer of apparently identical subunits, later confirmed by direct studies with human PA (4–6). It was established that $P_t$ 1–1 and $P_t$ 2–2 are the homozygous types resulting from two alleles designated $P_t^1$ and $P_t^2$. The mode of inheritance was autosomal dominant. Analogous studies on M. mulatta were simultaneously performed in another laboratory, with similar findings (7).

The phenotype and gene frequency in several populations of M. mulatta are listed in Table I, and show considerable variability. Furthermore, Weiss et al (8) have surveyed eight species of macaques and found the frequency of $P_t^2$ to vary from 0.05 to 1.0, the latter in M. fuscata, a Japanese species. In most cases, the phenotype frequency was in Hardy-Weinberg equilibrium, indicating no selection for homozygous or hybrid types. Although the possibility has been

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TABLE I

*Incidence of Prealbumin Polymorphism in Macaca Mulatta*

| Reference | Number of Individuals | Phenotype Frequency (%) | | | Gene Frequency | |
|---|---|---|---|---|---|---|
| | | $P_t$(1–1) | $P_t$(1–2) | $P_t$(2–2) | $P_t^1$ | $P_t^2$ |
| Bernstein et al (3) | 142 | 79 | 18 | 4 | 0.877 | 0.123 |
| | 49 | 90 | 8 | 2 | 0.939 | 0.061 |
| | 24 | 79 | 17 | 4 | 0.875 | 0.125 |
| Alper et al (7) | 23 | 64 | 28 | 8 | 0.778 | 0.222 |
| Weiss et al (8) | 7 | 43 | 57 | | 0.714 | 0.286 |
| | 25 | 80 | 20 | | 0.900 | 0.100 |
| | 21 | 90 | 10 | | 0.952 | 0.048 |

raised that physiological factors might affect the gene frequency (8, 9), there is no evidence that anything other than random genetic drift and founder effects are involved, as with most polymorphisms (10).

Some other primates have also been investigated. No polymorphism like that in macaques has been found in baboons (1), chimpanzees*, and orangutans*, but drills and mandrills have been found to have PA polymorphism (8). Recently, the opossum was also demonstrated to have a polymorphism in the major thyroxine-binding protein, a postalbumin, which was linked with a haptoglobin polymorphism (11).

There is no evidence that the PA polymorphisms so far described affect thyroxine transport. In M. mulatta, isolated $P_t$ 1–1 and $P_t$ 2–2 each contain a single binding site for thyroxine with identical affinity constants (12). It has also been shown that binding of RBP (the retinol-binding protein which circulates as a complex with PA) is unaffected by the polymorphism (12).

In man, an uncommon polymorphism of serum albumin—"bisalbuminemia"—occurs in several forms. Usually, the trait is heterozygous. In addition to apparently normal albumin, one of the two general types contains a slower-migrating electrophoretic band ("slow type") and the other a band moving faster than normal ("fast type"). There have been a few reports of thyroxine-binding studies with these sera, with variable results (13–17). Usually both albumin species in a serum bind thyroxine, but compared to the normal albumin, the abnormal may bind more (13, 14), the same (16) or less (17) thyroxine. Since albumin-binding is certainly not saturated in these studies, these results probably reflect variations in the affinity constant for thyroxine, but detailed quantitative studies have not been done. However, in view of the minor role of albumin in thyroxine transport, it is unlikely that these variations carry physiological implications. In the human fetus, a thyroxine-binding pattern resembling bisalbuminia is seen (15) but this is due to the presence of a fetal

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* Sera from 96 individual chimpanzees and 22 orangutans were examined by polyacrylamide gel electrophoresis using the technique described earlier (3). A single thyroxine-binding prealbumin band analogous to $P_t$ 1–1 of M. mulatta was observed in all cases.

"postalbumin" protein which is unrelated to albumin and which binds thyroxine.

Much more common than polymorphisms in man are genetic variants in which one of the transport proteins is reduced or increased in amount. Although such a variation could result from a polymorphism, it will be seen later that none has been disclosed. No genetically determined alteration in PA level, except in association with abnormal TBG in one family, has been described and we will be concerned mainly with variations of this type which affect TBG. We should first mention, however, that the rare trait, analbuminemia, obviously affects thyroxine transport since serum albumin is virtually absent. It is of interest that the major thyroxine-transport proteins, TBG and PA, share in the general elevation of many of the serum proteins which is seen in this disorder (18). Measurement of the free thyroxine level in serum has revealed a moderate elevation (18) the significance of which is unclear. It is tempting to consider this secondary to a change in circulating free fatty acids, since the latter can affect free thyroxine in some circumstances (19) but no evaluation of this or other factors has been made. Although these patients may have problems stemming from the lack of serum albumin, it has not been shown that the thyroxine transport changes are deleterious.

The first suggestions that altered TBG levels might be inherited came from the finding of sporadic cases showing abnormal thyroxine-binding capacity of TBG with no other cause apparent (20–25). The genetic etiology was especially likely in cases with no detectable TBG binding (21–23). The first report of multiple cases within one family, however, was by Beierwaltes and Robbins (26) who described elevation of TBG capacity in a father and daughter who were otherwise healthy. Beierwaltes et al (27) subsequently discovered more cases in relatives of the propositus. The mode of inheritance was thought to be autosomal dominant but, as pointed out later (28), was also compatible with an X-linked mode. In the ensuing years, studies of a number of families exhibiting elevated (29–35) or reduced (28, 35–47) TBG capacity were described. The ease with which these families came to light suggests that the traits are not at all uncommon.

The mode of inheritance may not be identical in all families, but the most extensive studies clearly showed or strongly suggested an X-chromosome-linked inheritance (28, 30, 32, 33, 37, 38, 43). In those families with deficient TBG, affected males had complete absence of TBG binding and affected females had a variable reduction of the TBG binding capacity, consistent with the Lyon hypothesis which states that female somatic cells are a mosaic with respect to expression of one or the other of the two X-chromosomes. Male-to-male transmission did occur, and affected males transmitted the trait to every female offspring. In elevated TBG capacity with a well defined or possible X-linked inheritance (26, 30–33, 35), the affected males exhibited a higher TBG value than the affected females. Average values for the hemizygote males are 2.7 to 4.5 times normal, and her heterozygote females, 1.9 to 2.7 times normal (35).

In one family, a unique finding of absent TBG in a female was described (47). She had a brother and son with similar abnormality, a daughter with low TBG and a normal mother and father. No adequate explanation for this occurrence could be proved, although several possibilities were suggested.

In several reports a search was made for association of the TBG abnormality with other X-linked traits. Thus far none has been found with color blindness (32, 37, 40, 43, 45) or with the $Xg^a$ blood group (32, 43, 45). Two interesting families have been described in which the probands were under medical attention for sex chromosome aberrations. Refetoff and Selenkow (40) reported a case of Turner's syndrome (XO) with absent TBG and an X-chromosome traced to a mother carrying the TBG defect. Moloshok et al (45) described a patient with ambiguous genitalia, one testis, and one streak gonad and a mosaic of XYY/XY/XO. The TBG capacity was reduced but not absent. Other affected males in the family also had low levels of TBG, so the failure to exhibit absent TBG in the proband could not be ascribed to X-chromosome mosaicism. As expected, the proband inherited the trait from the mother. Affected males had lower TBG capacity than affected females, and the authors postulated a probable X-linked inheritance differing from the one causing absent TBG in males, but perhaps an allele of the same gene.

Other families in which TBG is diminished but not absent in males have been reported (35, 36, 39, 42, 44, 45), and some of these clearly show X-linked inheritance. In some families an autosomal type of inheritance has been proposed (36, 39). Since no instance of male to male transmission has been shown, however, the question of the mode of inheritance in these families must remain open. The same may be said of families with increased TBG capacity in which autosomal inheritance has been suspected (27, 29).

Few other abnormalities associated with the inherited TBG defect have been reported. In one family, elevated TBG capacity was accompanied by a high incidence of simple or multinodular goiter (33). The goiter appeared to be X-chromosome linked but in a separate gene locus. In a second family (31) ectodermal displasia—itself an X-chromosome-linked trait—occurred but was considered to be coincidental. In another family absence of thyroxine-binding to PA was found (34) and in still others the propositus was under study for growth or mental retardation (34, 48, 49). The significance of these and other (35) associations is uncertain.

The mechanism by which the abnormality in thyroxine-binding is produced in these families has not been fully defined. We have spoken only of variations in thyroxine-binding capacity, since this is the way in which the defect is usually identified. This could result from an abnormal level in serum of otherwise normal binding protein, or from the production of an abnormal protein. Since normal TBG contains a single site for thyroxine binding, a mutation affecting this site would lead to an absence of thyroxine-binding (when examined by the thyroxine-binding capacity measurement) unless it affected TBG production or metabolism and did not competely destroy TBG binding. In the former case, it might be possible to identify TBG in serum by immunochemical but not by thyroxine-binding techniques. One report described a patient with absent TBG binding and a serum protein reacting with antiserum against TBG (50) but this evidence for an abnormal TBG was later denied (51). Presumably the antiserum was not specific. Two other reports failed to find immunochemically reacting TBG in similar patients (35, 52).

By similar reasoning, an increase of thyroxine-binding capacity produced by an abnormality of thyroxine-binding to TBG should result in a protein with two or more binding sites per molecule. No studies of TBG isolated from patients with this congenital defect have been reported, but studies using quantitative immunoassay showed that TBG capacity and quantity were directly related (35, 53). There have been attempts to examine various properties of the elevated or reduced TBG compared to normal (35). Although detailed molecular studies with TBG purified from such patients have not been done—and are necessary for a definitive answer—no abnormality of the protein or its peripheral rate of disappearance from blood has yet been disclosed. Furthermore, there is no evidence for an inhibitory substance in the serum of patients with decreased TBG capacity which could affect thyroxine-binding by normal TBG (40, 46) nor for the lack of such a substance in increased TBG capacity (27).

The decrease or increase of TBG binding, if accompanied by decrease or increase of the protein itself, could result from a structural gene deletion or duplication or more likely from a structural gene mutation which affects the rate of synthesis of TBG. In TBG absence, a frame shift mutation, for example, could reduce synthesis to zero. It is noteworthy that homozygous individuals with elevated TBG have a binding capacity which is usually more than twice normal, and can be as much as four times normal (26, 30–32, 35). The reason that the amount of gene product would be more than doubled if there were a gene duplication is not clear but could be due to a limitation in synthesis-control or product-degradation processes. There are very few other inherited abnormalities known to cause an increase rather than a decrease in the affected protein. An inherited increase in G6PD is also an X-chromosome-linked trait and it is of interest that this also shows an increase in homozygous individuals which is about four times normal (54). One other protein showing an inherited increase is plasma pseudocholinesterase (56), but this has an autosomal type of inheritance. In the case of G6PD Hektoen, a point mutation has been demonstrated (55) and several explanations for the resulting four-fold increase in enzyme have been proposed (55, 57, 58). Among these are that the normal gene product is a corepressor of transcription, or that the release, stability or translation rate of m-RNA is altered due to the base change in DNA or in RNA. A decrease in protein synthesis resulting from a point mutation can also be explained in an analogous but opposite way.

Another way in which the level of TBG could be affected is by a genetic alteration in a control step in TBG synthesis. One could postulate that the TBG gene is normally controlled by an operator gene which is in turn under the influence of a repressor gene. One could postulate further that the repressor gene is the site at which estrogens and androgens normally exert their presumed effect on TBG synthesis. It is then of interest to examine, in the TBG variants, the effect of these hormones on TBG levels.

If the deficient TBG syndrome is caused by a defect in the operator gene, estrogen administration should have no effect on TBG synthesis but a defect in the repressor gene manifested by excess function of this gene might be influenced by estrogen. Several males and one female with absent TBG showed no TBG response to estrogen (22, 28, 38, 47), and there was no response in an XO and an XYY/XY/XO individual (40, 45). In females, who are heterozygotes, one would expect a significant response to estrogen or pregnancy; it is surprising that the increases of TBG have usually been small (35, 38) or nonexistent (28, 48).

If the excess TBG syndrome is due to a defect in the repressor gene, it is possible that estrogens and androgens would no longer control TBG synthesis. In one female subject, estrogen had no effect but norethandrolone produced a fall of TBG level to normal (25). In a male and in several other females, estrogen did cause an increase in TBG, but the effect was significantly less than expected in normals (30, 33). Of course, a defect in the TBG structural gene could also affect the response to estrogen.

It is of special interest to examine the cortisol-binding globulin (CBG) in patients with the TBG variants. CBG has molecular properties very similar to TBG and it responds similarly to estrogens. It is possible, therefore, that CBG and TBG are controlled by a single repressor gene. In all cases studied, whether with increased (25, 30) or decreased (28, 38, 40) TBG, CBG levels were normal, and responded normally to estrogens with an increase of two- to three-fold. A normal increase of ceruloplasmin (25, 28) and testosterone-binding globulin (35) levels after estrogen was also observed. It is also of interest to note that only one family with an inherited deficiency of CBG has been reported (59). Unlike most TBG variants, the inheritance was interpreted as autosomal dominant with CBG level reduced to the same extent in males and females and responding subnormally to estrogen.

It is difficult to interpret all of the findings with respect to the genetic mechanism in these TBG disorders, but the best unifying hypothesis is that a structural gene located on the X-chromosome is the site of the abnormalities. It is of course possible that more than one mechanism is involved.

The altered TBG states have provided important evidence concerning the physiological role of this protein. Since subjects with absent or elevated TBG show no clinical evidence of hypothyroidism or hyperthyroidism, respectively, it must follow that thyroxine does not exert its action through formation of the TBG-$T_4$ complex. There are some reports (34, 44, 46) that the free thyroxine concentration is decreased in TBG deficiency or increased in TBG excess, but generally the available data strongly support the concept that the free thyroxine level is the factor which determines thyroid "status". The changes which occur in thyroxine kinetics (22, 25, 26, 30, 35, 36, 38, 40, 41) seem related entirely to changes in the extrathyroidal thyroxine pool. Thus TBG appears to play a passive buffering role in thyroxine turnover. In future studies it should be of interest to see what effects TBG absence or excess have on thyroxine kinetics in the microcirculation since the rate of dissociation from the transport protein may be important for hormone delivery to tissues.

From a practical clinical standpoint, inherited variations in thyroxine transport proteins are important mainly because they produce spurious effects on those thyroid function tests based on hormone level or binding in blood (48, 60, 61). Their greatest significance may lay in their application to studies of human genetics and protein synthesis since TBG variants involve the X-chromosome and are among the few traits which lead to an increased protein level.

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# Foreword

KURT W. DEUSCHLE, M.D.

Known throughout the nation and world as the "champion" of public health and preventive medicine, George James was identified with almost every "cause" in the interest of advancing and promoting community health. This memorial issue can only touch lightly on the multitude of issues for which he lavished his energy, thinking, and articulate expression, full of strength, good humor, wit, and charm.

Dr. James left his post as Commissioner of Health in New York City to become the first Dean of the Mount Sinai School of Medicine and President of The Mount Sinai Medical Center. He was keenly sensitive to the need of, and indefatigable in his drive for, excellence in education and training that would enable young physicians to assert leadership in the complex urban communities of today and tomorrow.

Although Dr. James gave The Mount Sinai Medical Center his most urgent and passionate commitments, he continued to find precious time for his basic interests in public health and preventive medicine at the city, state, national, and international levels. His peers everywhere looked to him for guidance and innovative ideas for the promotion of health care in the future. The diversity of Dr. James' professional interests and concerns spans a range of subjects, issues, and themes that do justice to the true "renaissance man."

Selected leaders in the health care field who worked with and admired Dr. James have written special essays on those topics which were close to his heart in his professional lifetime. The articles are presented in an order that George James himself might have used in the preparation of a lecture, that is, present the problems, discuss the issues, and offer some hoped-for solutions.

Dr. Deuschle, the Guest Editor, is the Ethel H. Wise Professor of Community Medicine and Chairman of the Department, Mount Sinai School of Medicine of The City University of New York, N.Y. 10029.

# George James and the Individual Man

DORIS SCHWARTZ, R.N.

As a local health officer and a public health administrator, much of George James' career was devoted to viewing the entire community as a patient. Concerned with providing services to whole populations, he was skilled in evaluating all that affected the search for health of an aggregation of people.

Yet George James never lost his awareness that a community was made up of a great variety of individual persons—persons with or without a so-called "significant other" to share responsibility for their care; persons with or without the need to act as the caring person for a spouse, mother, or dependent children. These were the persons who could (or could not) climb the steps of a bus to reach the clinic in winter; persons who could (or could not) carry parcels from the grocery store to their homes; persons who did (or did not) use judgment in living the life styles which facilitate first-stage prevention of illness.

This ability to see the minutiae of health care in two dimensions simultaneously—to be concerned both with persons and people—was one of the strengths which made James great. With people, his input was that of the scientist, the epidemiologist, the community planner. With persons, his input came from the heart.

He had the criteria of a professional, laid down by Follett: ". . . a foundation of science and a motive of service. . . ." The two components were inextricably melded in him. So was his concern for *all* of the stages of a health problem. These stages, James defined as "conserving what health is already present . . . giving special attention to high risk persons and groups . . . detecting pathology at the earliest possible moment . . . intervening in sickness with all that we know how to do . . . giving thought to the rehabilitation process from the moment the need for it occurs [and] for those for whom our knowledge has no answer at this time, preserving them from unnecessary suffering and permitting them a peaceful death."

The language he used was simple and pithy. Not the least of the strengths for which he ought to be remembered is "George James: Linguist." He expressed concern because "the horizontal patient gets far better care than the vertical one." He spoke of how the goal of each of the health professions was "to tip the scales in the patients' favor." That was the touchstone he always gave to students: ". . . all your knowledge and your judgment is to serve one purpose—to tip the scales in favor of your patient."

Students turned on quickly in his presence. They identified with the old lady he told of, who missed essential appointments at the hospital because she needed a podiatrist's care so badly that she couldn't put her shoes on to travel to the Diabetic Clinic. Students were with him when he said, "When care is good, it is concerned with all the stages of all the health problems of which this

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patient is a part . . . to be good, all persons living within range of a health facility must have access to its care."

James was always concerned with health education, putting his emphasis upon "helping people to learn" rather than on "teaching them." He saw education's goal as "helping people attain for themselves and for others, the best possible health." He would look at a room full of smokers, make a few dry comments on air pollution, then add: "Smoking—a portable form of air pollution."

His personal approach to health education was always the broad picture, then a light needling of his listeners, and the point slammed across unforgettably. With documentation.

As Health Commissioner he was personally responsible for educating all kinds of people about health: the mayor, the city council, the citizens union, the irate taxpayer. He did it with simplicity and charm, always emphasizing the cause and scope of a problem and the rationale underlying the recommended action. He pointed out the importance of having ambulatory care centers close to where the patients lived. He showed the need for them to adopt a more uniform system of classification and of standards. He documented the benefits gained by coordinating them with the community's other services. Finally, he stressed the advantages of making the back-up services "comprehensive," for example, of having on-site interviews, public health nursing, social services, rehabilitation, planning, all coordinated within "a single health care program." He understood and interpreted the importance of "continuity" in this health care scheme.

He was not always successful in his personal efforts at health education. For all his attempts to improve the reporting of venereal disease, little was accomplished during his tenure as Commissioner. Local medical doctors continued to treat syphilis without reporting it. Discussing this with a group of student nurses, on one occasion, he made the point that "health education is a complex thing with many hard-to-define components." When thinking about how to help people learn, "we need to consider the emotional stability of the target group, their permanency in the situation, their age differences (each age with special possibilities and special hazards), and the differences in their capacities—for example, intelligence or its lack, knowledge or ignorance, happiness or misery, economic and other power advantages of one form of behavior versus another."

He came on strong in his vignettes on the importance of not stereotyping people. His health education was strongly directed to the individual man, be he politician, fund-giver, or patient. He well understood John Dewey's comment: "Might as well say he has sold when no one has bought as to say he has taught when no one has learned." He thought most health professionals failed in helping people to learn because of "lack of appreciation of the opportunities for teaching, lack of knowledge of what to teach, or lack of motivation to do the teaching." These three factors were never wanting in him.

He was quick to note, and to help others note, the difference between "eligibility" and "help." "Eligibility" meant to him, in Harry Golden's words, "You're entitled." It wasn't much use unless help was there too. "Help," in James' way of thinking, was "what you need when you're in trouble and it comes from people who do for you the things that need to be done. Most of us," he said, "tend to overemphasize questions of payment for health services. We would do much better to start with the patient and the questions of how to provide what he needs . . .."

He was always concerned with how much of medicine and health work lies in the years before the patient visits the physician, nurse, or social worker with a set of symptoms. "Health care is concerned with seeing the patient as an individual, seeing patients before they have symptoms, dealing with patients on a prolonged, continuing basis, and maintaining professional standards of patient care."

He saw health care as "a ministry to society . . . Many of the major problems with which we are dealing can only be attacked by changing the patient's whole relationship to the world in which he lives and by altering social patterns."

He had a flair for putting himself in the shoes of the person in trouble, recognizing how great the need for a health worker to whom the patient could turn as a primary practitioner—not necessarily a physician. "The various problems to which man is subject are put into manila folders which represent specialists and the various specialty groups . . . the folders are kept in files in different buildings but the person whom they are about is one man . . . the manila folders can be brought to sit inches apart on the desk of one doctor but they may mean a trip of four miles with two bus transfers for a sick old man of 80 who is unfortunate enough to have something wrong with different organ systems. . . . If he has six organ systems affected, his clinic-going may become a full-time job. The clinics themselves are often located not in relation to the old man's needs but according to who provides the funds for what disease headings . . . now, the old man still has a choice. He may, and often does, choose not to go to any source of medical care."

"Human beings," said James, "are difficult enough to understand when one sticks with them. When they are shuffled about, it is quite impossible . . . services should be packaged so that they can be utilized to the fullest."

A single paragraph of James' health teaching may contain an Alfred North Whitehead thought on lack of vision among educators and a line of Louis Untermeyer's poetry on living in an age of transition. Poetry, philosophy, and a realistic awareness that "we do not know as much as we should about how to get people to change their habits" may be melded into wisdom from him. He acknowledged freely that he was puzzled by what next step to take. "Should we frighten people, shame them, flatter them, enrage them? . . . Perhaps the main line of attack consists of convincing the patient to use his will power to change his life, to prolong his life, and to better it. . . ."

Whether teaching or preaching, helping people to learn, or investigating new methodologies of problem-solving, this was the common denominator of all James' efforts in health education: "to improve what is, so that what is to be will be better."

# The Role of the Medical School in the Delivery of Health Care

HANS POPPER, M.D., PH.D. AND REBA NOSOFF

## *1. The Problem*

The primary purpose of a medical school, old or new, is to train physicians. The argument concerns the "type" of physician. Today, most recent graduates remain and continue their education during the house staff years under medical school and hospital auspices, and these increasing numbers of graduates in training are considered a responsibility of the medical school which guides them thus in their career choice during student and house staff years. This combined product of undergraduate and house officer training can become any of the following:

1. A primary care physician or general practitioner, or, as is now often the case, a combination of internist and a specialist, particularly a pediatrician;
2. One who is devoted to the organization of medical care in community medicine, administrative medicine and even preventive medicine, with or without specialty training in, for example, internal medicine;
3. A specialist in one field of medicine, giving secondary and tertiary care;
4. A clinical scientist who, in addition to a tertiary care specialty, concentrates on research; and, finally
5. A basic scientist who may replace house staff activity by graduate work.

The decision as to which track to choose is left to the student but guided by contact with senior physicians as well as the changing fashions in public opinion.

There is a feeling in the nation at large of a maldistribution in these various categories, particularly a lack of the first group, the primary care physicians. And throughout the nation, the medical school influence determines health care by the category of physician it produces.

But if the "primary" purpose is to educate, the "ultimate" purpose of a medical school is to promote health. "No part of higher education in recent years has shown the capacity to reform more effectively than have the medical schools," said Dr. Clark Kerr, Chairman of the Carnegie Commission on Higher Education (1). Adaptation to science, revised curricula, emphasis on sociologic problems, earlier student contact with patients, three-year degree programs, larger numbers of women and minority students, and increased cooperation with local hospitals and city health departments are illustrative, added Dr. Kerr, of the many efforts to change and adjust. Medical schools have become

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models for self-reform successfully undertaken, models for the rest of higher education.

In addition to providing the "type" of physician, the medical school is being charged with the responsibility for developing a system of health care. This task is considered a scientific problem but is more often carried out by trial and error than by rational, goal-directed research. The hospital with all its satellite institutions serves as laboratory for both education of health workers and development of a health care system.

Medical school educators are faced today with the problem that health care has developed without planning. As disease is not planned so is the system treating it without planning. As Perkins (2) so aptly put it, when students leave the school of agriculture they are trained agriculturalists who have also been provided with a method for practical use, and they have learned it on experimental farms. However, the school will not take the responsibility of conducting large scale agriculture activities outside of demonstration leaving that to the farmer and county agent.

The greatest problem, however, is the size of the population in the "laboratory" for demonstration and teaching. Some demand that the medical school take responsibility for the health care of their region, independent of the size needed for the "laboratory." Others feel strongly, analogous to demonstration of the agricultural schools, that the size of the population served should be determined by the needs of the "laboratory." The conflict depends on cultural, ethnic, and sociologic local circumstances; for example:

In Beersheba, Israel, a school has just been founded where the Chief Administrative Officer of the school is the chief health officer of the region.

George James was one of the public health leaders in the nation, not only recognizing but eloquently verbalizing these views. As the public slowly awakened to the problem of unequal distribution of medical care, George James, in his historic role, became the interpreter for these "strongly felt, but poorly articulated" feelings. Through years of government and public health work, he understood the great responsibility of the health professional in this country. He felt that society had the right to ask for low-cost, accessible medical care while being taught to recognize and request higher quality, more effective care. The current status of medical knowledge permits us to do fairly well with the diseases which were the scourges of the past, but we are seriously lacking in weapons against our present leading causes of death and disability. What is more, we do not yet know the best pattern to follow for the most effective delivery of medical care. But one thing seems clear. The medical care "crisis" will not be solved by feeding numbers of new young physicians into the communities as an attempt to meet increasing demands of the population with the increasingly costly techniques of present-day curative medicine.

When new systems are being developed in the health care system in which nonphysician health workers take an important part, the medical school will take the responsibility for training these members of the health care team, enlarging further the role of the medical school.

Thus, the mutual relationship between medical school and the health care system raises a series of problems:

1. The education of the "type" of physician and nonphysician health care worker;
2. The development of a health care system by the use of scientific methods;
3. The use of the health care system as a "laboratory" to train the health worker, Ph.D. candidate, and others;
4. The provision of health care to those patients who are participants in the demonstration and part of the health care "laboratory."

### *II. History of Problem*

The history of medical education in America is interesting because it was challenged by abrupt changes. One occurred during the latter part of the last century, at which time apprentice teaching, with emphasis on the care of the individual patient in his home, was replaced by medical school/university-type teaching, an "academic" revolution. While it improved the basic knowledge of the physician, it took away attention from the individual patient at his home and concentrated physicians in hospitals.

Even in colonial times in this country, the first medical schools were a part of university settings. The University of Pennsylvania School of Medicine was originally the medical faculty developed by the College in Philadelphia in 1765. The College of Physicians and Surgeons of Columbia University was originally the Kings College Medical School founded in 1768 in New York. In the nineteenth century the university-affiliated medical school became the most desirable pattern for American medical education, seen as a community of scholars prepared to teach in all areas of human endeavor.

Between 1900 and 1910 another revolution occurred spearheaded by two main events: the founding of Johns Hopkins Medical School after the pattern of German Universities and the Flexner report in 1910 which made biology the cornerstone of medical education in America. For instance, materia medica was replaced by pharmacology which stressed action of drugs in the body rather than for their description and their subjective effect. The Flexnerian model brought American medicine to great eminence and the successes in health care, prolongation of life span, for instance, led to wiping out infectious diseases for the most part, but the Flexnerian principle maneuvered the educational system into a lock-step arrangement standardizing the product. It was excellent in biomedical activity, developing a scientific method which culminated in molecular biology representing a triumph of correct thinking rather than detailed observation. However, the scientific method resulted in some alienation of the physician from the patients' personal problems by emphasis on the disease and on specialization. This thinking, developing from the "biologic" revolution, was on the American scene until the middle of the sixties.

The third most recent and also rapid change could be characterized as the "sociologic" revolution. Our society has expressed the desire to supplement biologic excellence with concern for the mind and dignity of the individual whether he be of the affluent or disadvantaged population. This influenced medical teaching to the point where sociology, ethics, economics, and social anthropology became an essential part of medical education, though previously not considered basic to medicine. At the same time, the community became an important teaching laboratory for the medical school.

During the Flexnerian period, the hospitals in this country were undergoing a similar change. Their origin in the early American colonies portray the hospitals as a combination of nursing home, shelter, and almshouse—certainly not a place primarily for the sick. Later, hospitals began serving only the sick and mainly the indigent sick. It was at the turn of the twentieth century that the hospitals began serving the "upper classes" and, simultaneously, the hospital assumed an increasing role in medical education. Currently, it is the prime organ of clinical education. There is an increasing demand for hospital beds, stimulated in great part by the various health insurance plans. However, the development of ambulatory local health facilities is correcting the situation and also bringing the student into the community.

In addition, the structure of the population has changed to include increasing numbers of aged people. These people keep to themselves, rarely going out because of fear or disability. They eat poorly either because they are unable to shop for food or, as is often the case, they do not have proper dentures; they often need corrective glasses and do not see well, and they require the services of a physician more frequently, but they seldom see one. So the role of health care administrator and educator has altered radically along with medical care itself, for it is now necessary to know how to administer neighborhood health centers, group practices, multiphasic screening programs, extended care programs, drug-abuse and alcohol centers, etc. He is no longer confined to the four walls of the hospital or school.

### *III. Proposed Solution*

The purpose of medical education is to develop a product that is socially useful, and the goal both of old and new medical schools is to produce physicians whose training is consonant with the future needs of society. The medical profession, like other elements of our society, has begun stirring with the vibrations of its students (3). The word "relevancy" as opposed to "tradition" is ever present and has, in fact, two parts. The first is the applicability of certain details of basic science information to the effectiveness of patient care. The second has to do with the relevance of the entire medical center and the medical profession itself to the total community in which it works. The stimulation of student inquiry is mandatory to fight teaching complacency. There is, indeed, a deep, new, unprecedented, worldwide generation gap, and once this is firmly established in the minds of both the young and old, teacher and student, communications can be resumed. Nowhere in the world are there elders who know what the children know, no matter how remote and simple the societies in which the children live. There are no elders who know what those who have been reared in the last 20 years know about what the next 20 years will be (4).

It will be the first and most important function of medical educators to predict the medical system of the future, anticipate the areas of significant change, and educate toward that end. At present we can detect trends in almost any direction, but to accept one trend valid above the other is a function of the politician educator, not of the scientist-educator.

When the Mount Sinai School of Medicine was in the planning stages, those of us who planned for the future were rich in dreams and poor in experience. Perhaps because of naiveté, we dared to present schemes which had not yet met the test of student and faculty resistance. However, our philosophy for the new curriculum took into account the newer trends and goals in health care and life sciences as they affected medical education. Education, after all, is a question, not a scientific problem answered by inductive reasoning, as is research. Research, therefore, may lead to irrefutable answers in medical education, that is, at its best when following the example of Socrates, i.e., through dialectical exchange. Physics you cannot argue; biology you "may" argue; but in education, where there is morality and conscience, you must argue.

The philosophy behind the design of the Mount Sinai School of Medicine considered prediction of the future as the most important factor in shaping the curriculum and outlining research problems, both in biologic and sociologic areas including community medicine, a special concern of George James'. Many of the key points stated in the original concept are even more applicable today. For instance, the increased use of quantitation in biology and medicine has indeed changed methods of medical practice. The increase in the amount of medical information makes it impossible for an individual to retain all essential facts. Technologic sophistication in medicine is rapidly developing. The importance of behavioral and environmental factors is now recognized in the prevention, diagnosis and management of disease. Geriatrics has become an increasingly serious problem as predicted. And the importance of recognizing disease in the preventable, presymptomatic state has come to the fore. History clearly indicates that a major disease has rarely been controlled until we have learned how to attack it before the occurrence of its symptoms (5).

It is important that the medical student understand (and also society, and the government as its arm) that our only hope for an impact on the leading causes of disease and death lies not in more physicians, nor increased health manpower, but, in fact, on research. George James often said that the question: "How much can we afford to spend on research?" should be rephrased to: "How long do you wish to spend money for ineffective answers instead of diverting more of it to a search for the correct ones?" (6).

The present trend is to take brilliant people and go into a new field of health care delivery without thinking through its organization and implications. What is really needed is a system of cost accounting to determine how much every school and every man contributes to research and education. So far we have received only artificial figures which add to confusion and empty motions. It is interesting to inquire into the social processes through which individual scientists are evaluated and to discover why some scientists rise quickly to positions of eminence and others remain relatively obscure.

Medical education will have to extend to many areas, and physicians, of necessity, will have to continue their education. In time, reexamination in the medical specialties may become the rule in order to retain their status. Postgraduate education continues to play a role throughout the physician's life.

We stated in 1963, and subsequently published (7), that future physicians will be called upon to be superb technicians steeped in quantitative biology, physics, and engineering, to be equally equipped for treatment and for prevention of disease, and to be cognizant of the social, economic, and psychological problems of their patients. While we may be forced to permit him to be a specialist in a narrow area of the body, he will have to remain a generalist as far as the mind, family, and ethnic background of his patients are concerned; but we subsequently realized that this physician may be so concerned with the structure of nucleic acids and the morphology of cancer cells that he might not be deeply involved in the community side of medicine. It was George James' feeling that we need some of our superb scientists to be dedicated to community medicine (8). "Other areas of medicine attract the brains and the sparks. The lure and drama of the operating room are well known," he said. "But," he added, "we must give more exposure to community medicine in our medical schools to provide a reasonable basis for choice between it and the scalpel."

Thus, while neither experience nor quantitative data are available to design the arrangement for the future, motivation will have to be based on common sense and on the principle of excellence in developing a medical educational system which serves the multiple purposes of medicine for the future. Implementing the founding philosophy, under the guidance of George James, Mount Sinai School of Medicine became a pluralistic school and will, hopefully, remain so in the future. As long as biology remains the cutting edge in research to detect and cure disease, excellence in biologic training will remain mandatory for any product of the school and, particularly, for the basic scientist and the specialist who are and will be the most numerous products of the school. However, concern for the individual patient's problems, aside from the biologic aspect of the disease, should be carried out with similar excellence which applies also to the specialist giving tertiary care, even in the ICU, who should be imbued with consideration for human concern, difficult though it may be in the drama of rapid decisions.

When we speak of balance it concerns the aspects of the consideration of the community at large which is so visually reflected in the ethical considerations which go into human experimentation where drug therapy or untried surgical procedures like transplantation may benefit other members of the community, particularly future generations, more than the individual patient. Balance of these conflicting demands results in solution, if tempered by excellence, not only for the medical school years, but also for the house staff years and even for the organization of the house staff itself. And this balance as a goal is the most important impact which medical education can make on the health care system. Thus, if we accept this balance and excellence as the key words, the school is not only the "laboratory" to develop it but the judge to decide on it as well.

### *IV. Difficulties in Solution*

Contemporary medical students are concerned with "social relevance" and demand that the medical curriculum rapidly move in the direction of satisfying the expectations of society. The changing role of medical schools in the health care delivery system surely affects medical education, but whether this will be for the better remains to be seen. A decision must be made as to whether we are going to teach medical techniques alone or also emphasize disease control. Indeed, the old family physician reached much further into community medicine than our physicians today as he made house calls and followed the families for decades. We are bringing back this concept into medical education, but on a much more sophisticated level. The students are exposed to the new community medicine so they will understand its importance along with obstetrics and surgery. Then a choice can properly be made on a career for his future.

Dr. John S. Millis, author of the Millis Report on the graduate education of physicians (9), expressed the belief that the group of people who can most influence the quality of life are the learned professionals. Therefore, the education of the health professional is in the highest public interest. The role of the health professional today, however, is frustrating to say the least. Society has invested heavily in his training, and competence in his field has made high quality care of great relevance. But the definition of relevance by that same society around him differs greatly, and he finds himself blamed by public opinion for the health crisis. Personal problems facing individual physicians and scientists are acute. Reckless use of scientific findings which, in themselves, are not inherently good or evil, plus the overwhelming desire to know and the possibility of extending life, has created conflict between the scientist's action and the mores of society (10). As an example, investigations are continuing on the ethical problems of transplantation, from the viewpoint of deciding in what part of the body reside the most unique characteristics of an individual—his identity. It is generally agreed that limb amputations, from a social standpoint, do not alter the patient's personality; neither do transplanted kidneys, hearts, livers or lungs. Therefore, the uniqueness of the person resides in the higher centers, the brain.

Our ability to grow normal human cells in culture presents a number of biological as well as philosophical problems. Genetic research made possible by the availability of cultured cells may be vitally important to many individuals. But the decision as to who will direct this genetic engineering, and in what circumstances it should take place are problems which must be dealt with.

So, we are faced with conflicts: the ethical conflict between the single patient and community concern; and the conflict between biology and community medicine which is mainly manpower and financial.

Against this background of intellectual and sociologic revolution, and against the question of "relevance," any prediction regarding the future practice of medicine would appear naive within a short period of time. Moreover, doubt has arisen that the most effective key in understanding the cause of disease is biologic. There is strong suspicion that emotional, social, and other environmental factors explain why some are afflicted by a disease while others are not. It becomes increasingly clear that exposure to injurious agents and genetic predisposition, by themselves alone, do not determine why a person becomes ill. These ideas supported by statistical studies open up new horizons as to etiology and incidence of disease not readily understandable in terms of biologic, or even psychobiologic, principles.

### V. *Prediction of the Future*

Teaching concepts in the medical schools are rapidly changing, and the physician of the future will have to develop attributes previously not taught. The Mount Sinai Concept (11) considers medical education in the definition of a tripod requiring balance* and embracing the following aspects:

a) Emphasis on quantitative biology in medicine which trains the physician to become an excellent technician equipped equally to treat and prevent disease.

b) Concern for the "whole" patient with his mental and physical problems as well as his family. Here the student is given further education and better exposure to teaching in sociology, psychology, social anthropology, economics, ethics, and philosophy of science.

c) "Community Medicine" teaching, that is, interest in society as a whole and dealing with prevention of disease and logistics of patient distribution.

In all aspects of the "tripod' 'the medical students will have to work with allied health professionals and, for problems in quantitative biology, will have to depend on the non-M.D., basic scientist, ancillary technician, and the biomedical engineer. In the areas of patient concern he must work with the behavioral scientist and the sociologist.

In addition, there are three trends in medical care and teaching for which we should be prepared. (12) They are not necessarily listed in their order of importance:

1. As society becomes more interested in medicine and the best possible health care, the administration of health care becomes more sophisticated and the responsibility tends to shift from the physician to the social scientist. The social and governmental sciences are policy-making and there is a reluctance as well as inability on the part of many in the medical profession to contest these decisions concerning medical problems. What is more, the government-social

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* While we speak of balance, it is important to add that in this paper, the aspect of Community Medicine has been emphasized out of balance as a tribute to George James who was brought to Mount Sinai to help develop a Department of Community Medicine during the time he was President and Dean. The selection of a public health man who was not in the main line of academia was a deliberate step by the institution to emphasize community medicine.

scientist will be more concerned with the interests of society as a whole rather than with the individual patient. Therefore, the importance of disease and its incidence and impact on society become related to the time it occupies in the academic curriculum.

2. The concept of regional centers with its hierarchy of local satellite units may change the relations between patient and physician, the relationships among physicians, and the structure of medical education and practice in the future. The private practitioner might play a lesser role because the best facilities in patient care exist in a center. The idol for a budding physician who wants to specialize in one field will be a new breed of medical leader, a specialist who combines clinical excellence in a circumscribed field with an understanding of basic quantitative problems in this field, and conducts research in it, but remains concerned with the personal problems of the patient and the needs of the community.

3. The development of computer techniques may alter the practice of medicine on different levels. While serving primarily as a labor-saving device it will facilitate diagnosis and monitoring of therapy and also allow the synthesis of new information. The problems inherent in the latter are so far great, but it is difficult to translate thought and impression into computer language. Success in this area will modify the course of medical teaching more than any prior development.

## *VI. Outlook*

The consumer in American medicine is becoming increasingly sophisticated in recognizing when medical care is of high quality in the medical center as well as in his home. We realize that we are confronted with occupational diseases and hazards, injury prevention, alcohol and drug-abuse problems, pollution of the environment, etc. This has prompted medical schools and other health care agencies to place special emphasis on the environmental health sciences. Nevertheless, with all its faults, the teaching hospital of today is still the site of the highest quality in medicine and of the greatest concentration of medical brainpower.

A medical school, in reality, is part of several communities: the near community geographically surrounding it, the regional, national and world community, and that other community consisting of the people who will be alive after we are gone. They are the ones who have most to gain or lose by what our graduates do.

Part of our job is to reach the intelligent layman who will make important medical decisions. They are found in state legislatures, on hospital boards, in voluntary health organizations, and elsewhere in the health establishment, but also in consumer organizations.

We have to develop a number of first-rate medical statesmen to work with the legislators and others in planning health programs and preserving federal and state aid for research and training. Perhaps only a small percentage of students would be suitable for such a role but we need to spot them and prepare them as well as to provide the necessary training.

Socrates wrote that wisdom consists of our knowing finally that we know nothing. Inquiry then must lead to knowledge. Health educators cannot be content to offer the future the inadequate medicine we use today. The way to avoid that is by learning to do better. This is the function of a medical school and it will help American medicine retain world leadership.

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# The Mass Production of Excellence

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One is tempted to punctuate the title of this paper with a question mark. The conflict implicit in its statement is, in fact, a question which constantly challenges those who participate in public and private discussions of the current "health care crisis." In my view, it is a conflict which should be minimized since it can be argued, even demonstrated, that it is possible for well-integrated, properly motivated teaching hospitals to deliver "the best" medical care to the great mass of our population.

To excel, of course, means to be significantly better than average, and it is asserted by some that if the bulk of our population were to receive the type of care that can only be given by exceptional physicians, these doctors would be impossibly overworked. Conversely, it is said, it is equally impossible to conceive of a situation in which all physicians are equal to the best.

Let us examine the first of these assertions. We must consider whether and how often patients actually require the services of an unusually qualified physician. It has been estimated that 85 percent of patient visits to physicians' offices are for trivial illnesses with no serious implication and that, indeed, are self-correcting; the physician's job here is one of symptomatic relief and reassurance. Still, there is general relevance in this type of primary practice, since the threat to life and well-being implicit in the appearance of any untoward symptom does create an understandable anxiety in the patient.

Accepting this description, we might ask why we continue to prepare specialists in all fields at an extremely high rate. Since even those internists and pediatricians who accept the role of primary practitioner are really prepared to handle esoteric and unusual illnesses which they will see but rarely in their practices, one cannot help but question the real value of overly sophisticated preparation of the primary physician.

The classic example is the continual production of excessive numbers of general surgeons into a medical economy which is characterized by excessive surgery when compared with the experience of Great Britain as well as that of the Kaiser Plan. This suggests causes and motives other than the avowed one of preparing the numbers and types of doctors that people need to be assured of optimal care.

Even if the resultant distribution of medical manpower by type and location does not meet the perceived needs of the population, one need not infer that this is a product of venal motivation. A major underlying cause is the progressive growth of the technology of medicine which has forced the concomitant differentiation of the physician. In order to apply each newly developed technique, a

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group of physicians must master it and, in turn, teach it. Being limited in number, the economics of scarcity sets a high value on their services. They are rewarded by professional status, as well as a high income and enjoy the personal self-imagery which grows out of performing a useful, life-saving function while being able to avoid the more boring aspects of routine medical practice. In turn, students are attracted to them and the intellectual challenge of teaching further enhances their satisfaction. In addition, it must be noted that students undertake much of the repetitious work characteristic of study and research.

As students mature, however, they move away from the primary teaching hospital where the techniques had been developed and begin to apply them in community hospitals. Since World War II there has been a tremendous flow of well-trained physicians in all the specialties to the suburbs along with the middle-income population who can afford to purchase their services. Being intelligent, imbued with the advantages of working in a teaching hospital environment and desirous of having the assistance of residents-in-training in their practice, they in turn have convinced hospital managements and accrediting bodies of their qualifications to head training programs and they have begun to replicate their kind.

The key point here is that this process, which has led to the excessive production of specialists, has occurred with no regard to national need. Rather, it was the local hospital situation which led to the proliferation of approved training programs so that we find a third of all house officerships are now filled with foreign-trained physicians. One cannot help but wonder whether hospital care would not be improved if practicing physicians in such situations gave up their training programs and assisted each other in the care of their patients.

This description of how our present distribution of medical manpower came about illustrates a cardinal point: The conflict between appropriate national goals and the goals of the individual hospital. Similarly, examples could be elaborated which would demonstrate the conflict between the community's goals for its hospitals and those of individual physicians; between the urge to satisfy the curiosity of individual researchers and the need for answers to specific health problems. This is not to say that any particular set of goals is wrong or right or that they are necessarily in opposition. But these differences exist and must be recognized if any system for the provision of medical care, for the teaching of medical students, and for the pursuit of research is to work efficiently and harmoniously.

Given these divergent goals how can rational systems be developed? Typically, when the desirability for change achieved widespread recognition, all eyes focused on the major medical institutions where such change is usually effected. The teaching hospitals were expected to add to their already large service loads even greater numbers of patients for whom they would be expected to provide total service. This did not prove successful for several reasons. Essentially, funding was inadequate and the teaching hospital which is the quintessential center for tertiary care, was simply not well organized for the provision of primary care.

The strong departmental organization which prevails in such hospitals and which was created to provide patients with uncommon illnesses with the most advanced technology developed in an atmosphere of teaching and research does not readily lend itself to the provision of total primary care. Accustomed to receiving their professional satisfactions from the acceptance of their original work by fellow experts in other centers, these institution-based specialists consider the gratitude of satisfied patients as a lesser order of psychic income.

The full-time staff is particularly troubled since they see their colleagues in private practice earning much more money than they do. They see themselves as being denied the tangible evidence of patient satisfaction which is more readily available to the private physician.

Apparently defeated in their perception of teaching hospitals, those who would solve the nation's health problems in radical fashion then undertook to organize neighborhood health centers which were to be deliberately divorced from the teaching function. The enthusiasm for something new has attracted idealistic young doctors, but, if these centers remain apart from teaching hospitals, the skills of these physicians will soon become outdated. The attractiveness of these centers is further diminished by the excessively high cost per patient.

How then shall the care of large numbers of patients be arranged? Shall we develop a totally publicly operated system? The history of New York City's municipal hospital system with its dependence for professional services upon affiliations with major teaching centers argues against this approach.

Shall we simply make sure that each person has adequate health insurance and ignore efforts at organization? The inflationary results of Medicare and Medicaid prove this to be unfeasible.

Shall we insist that each person become a participant in prepaid group practice? The number of people who opt out of such schemes is proof that many do not find them acceptable.

Or shall we offer a variety of plans and allow people to make their own choices? The political and cultural history of the United States will permit no other direction.

The major teaching hospital can provide large volumes of care to people of all economic levels who will come to it for primary as well as specialized treatment: 1) by assuring that it is a referral point for tertiary care from a network of affiliates as well as by the individual reputations of its medical experts; and 2) by giving its medical staff members an opportunity to share in the financial rewards of practice to some degree and encouraging them to practice actively. A plan which provides tangible rewards for patient care will help to balance the psychic income of research and teaching (and add to their tenured incomes).

There are those who think that all physicians should be salaried. If they were and if they worked the usual 40-hour week of the salaried practitioner, this country would need 50 percent more doctors, since the average physician now works about 60 hours per week.

Another technique for strengthening total patient care is to develop interdepartmental groups of physicians, even at the possible expense of the departmental chairman's authority. Facilities and fees shared by men from different departments will help to ensure responsive care by small groups of doctors attending numbers of patients small enough so that the physicians and their patients can come to know each other and to interrelate as human beings.

Finally, a more significant role for the institution per se is inevitable. Physicians gaining greater satisfaction at the hospital and greater identification with it will be more responsive to the insistent demands on the institution by groups of patients. These can be the desire for a new service facility or even a proposal to reorganize the delivery of medical care by the institution. Spokesmen will be able to reply effectively and definitely to such expressions if assured of the support of the appropriate members of the medical staff. This institutional responsiveness goes beyond medical care per se; as the public focuses its demands, for example, on an increased number of students or for some specific disease-target of research, the spokesman must be able to provide assurance that legitimate demands will be met.

Yet, in hearing the cries of people for change, a delicate balance must be struck. Most organized demands focus on short-term goals; open a clinic, admit more students, find a cure for some disease. The essence of the excellent institution is its commitment to long-range goals: to basic research, to the preparation of superb physicians, to integrity in the search for truth, to a stable society.

It is the balance between responsiveness to short-term goals and dedication to the longer-term aspirations that defines an institution. To avoid the first is to invite revolution; to ignore the second is to assure mediocrity.

The successful institution defines its role and enunciates it effectively. Aware of the intrinsic diversity of goals among its staff, its trustees, its community and the society in which it functions, a synthesis of purpose is arrived at. This synthesis may often be unarticulated, but must at least be felt and accepted by all who relate to the institution if it is to achieve true viability.

Given an institution's generally accepted basic concept (even if the concept is no more than an acceptance of differences) and a long-term commitment to valid goals, an institution and its people will find their satisfaction in a common striving to attain these goals. The clearer the goals, therefore, the greater will be the closeness of kinship and the warmth of camaraderie among the institution's staff members.

If one of these goals is the production of a large volume of high quality care for those who need it, the internalization of this goal by the staff members will help to make it a self-fulfilling prophecy. There are few greater satisfactions available to man.

In many ways the late George James was the exemplar of this synthesis of purpose I have referred to. It was perhaps his most distinguished genius that Dr. James was so well able to conceive and to articulate the many goals of Mount Sinai and to inspire the faculty and staff and, indeed, all at Mount Sinai who shared his vision and his aspirations to achievements of the highest order.

# The Relation of the Urban Environment to Health: Implications for Prevention

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Perhaps, of all the attributes that George James can be remembered by, the most enduring and persistent is his strong and unwavering commitment to the ideal of prevention of disease. Unfortunately, while there has been widespread agreement that this is an ideal that should be strived for, our "reach exceeds our grasp" insofar as nearly all the diseases that plague a technologically developed society are concerned. Implementation of James' ideals has lagged far behind our desires.

To a large extent this state of affairs stems from a neglect of some of the principles of prevention which, despite James' teaching, have not found their way into practice. Of these principles, perhaps one of the more important is the realization that, with few exceptions, disease has not been prevented by finding and treating sick individuals. Typhoid fever was not controlled by finding sick cases of typhoid, but by purification of the water supply. We did not reduce infant mortality by finding sick children, but by pasteurizing the milk supply. The message is clear; with the exception of those few diseases susceptible to immunization, our major victories in prevention have occurred when we have been able to modify those environmental factors that facilitate the occurrence of disease.

The question of concern then is what are the important environmental factors associated with our modern "plagues" (cardiovascular disease, neoplasms, arthritis, diabetes, behavioral disorders, mental disorders). The thesis advanced in this paper is that the categories of environmental factors currently suspected as being determinants of these diseases are inadequate to explain their occurrence. In particular, the environmental factors offered as reasons for the appalling state of health of the inhabitants of our inner cities do not adequately explain the existing phenomena. We need to focus research on other categories. This is especially true when we focus almost exclusively on such physical and physicochemical environmental factors as quality of housing, air pollution, and physical density.

A review of the literature of the relationship between these urban environmental factors (particularly the quality of housing and crowding) and health status reveals considerable uncertainty and contradiction. Since 1920 some studies have shown a relationship between poor housing and various indicators of poor health, others have shown no such relationship, and yet others an inverse relationship. Poor housing has been related to higher overall mortality rates (1), to higher infant mortality rates (2, 3), to higher mortality and

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incidence rates of tuberculosis (4–11), to the incidence of meningococcal disease (12), to digestive diseases (8), home accidents (8–13), anemia (14), and to higher hospital admission rates for contagious and nutritional disease (15). In addition, beginning with the classic study of Faris in 1939 (16), a plethora of investigators have shown a relationship between deteriorated neighborhoods and mental disorders, particularly the major psychoses (17). Finally, the relationship between slum conditions and violence, drug abuse, and alcoholism is too painfully evident at this point in history to require documentation by formal research.

In contrast to these studies, other investigators have been unable to find any correlation between quality of housing and health of the inhabitants. Brett (18), McKinlay (19), Mackintosh (20), McMillan (21), Lockhart (22), and Holmes (23) could find no relationship between crowding in households and tuberculosis; rheumatic fever and streptococcal illness did not occur more frequently in adequate than inadequate houses (24), respiratory illnesses were no more common in crowded than uncrowded conditions (25) and morale and "general adjustment" were found to be no better in slum dwellers following rehousing than in those living in the original slums (26). M'Gonigle's study in 1933 (27) showed an actual increase in death rates following the rehousing of a slum population from an "unhealthy area" to a municipal housing estate, and Martin et al (28) showed an increase in neurosis in inhabitants moved from inadequate housing to a new housing estate.

In part, these conflicting findings may be due to inadequacies in the research design employed in many of the studies. These include inadequate, or in some cases the complete absence of, controls, problems of selection, varying definitions employed to categorize the quality of the housing, and varying degrees of completeness and accuracy in the ascertainment of health status. In addition, few studies have attempted any long-term follow-up, and if the health changes are of short duration (for example, following relocation), studies conducted at different points in time following the event might be expected to produce discrepant findings. Of perhaps greater importance is the fact that in the majority of the studies, poor housing (however measured) is so closely correlated with other environmental and personal variables that it is difficult, if not impossible, to determine whether it was the quality of the housing per se that was responsible for the relationship or the cluster of the correlated variables.

Even when strenuous attempts have been made to overcome these methodologic and conceptual problems, as in the study by Wilner et al (29), the role of the housing itself as a determinant of health status remains unclear. As a result, an increasing number of investigators (30–38) are beginning to stress the point that the processes through which housing may be linked to health need to be examined and that these processes, which may vary for different health conditions, are likely to be more complex than was originally suspected. This approach suggests that the link between housing and health may be quite indirect, and that unless these intervening processes are understood, changes in the physical structure of the housing alone may have no effect on health if such a change does not simultaneously affect these factors.

### *Processes Linking Housing to Health*

There can be little disagreement that certain health conditions are directly determined by the physical structure of housing. The relationship of peeling lead paint to lead poisoning, of dilapidated structures to home accidents, the effects of inadequate heating, broken plumbing systems, the presence of insects and rodents, stagnant water in cellars, and inadequate waste management and disposal are perhaps self-evident and hardly need to await further research findings before action can be taken. Once the area of concern extends beyond these issues, however, the need for clarification of concepts and new research strategies becomes evident. This is particularly true insofar as infectious diseases (especially respiratory infections), the chronic diseases, and mental and behavioral disorders are concerned.

One of the more widely held and cherished notions in medicine is that the spread of infectious disease is facilitated by crowding. This assumption underlies many of the research endeavors seeking to establish a relationship between housing and health, and has been accepted as a truism by policymakers. There is little question that under certain circumstances crowding may be related to an increased incidence of communicable diseases, but, as reported above, under other circumstances no such relationship has been discovered. In fact, in two relatively recent studies of tuberculosis the highest rates were not found to occur among the most crowded individuals but among the social isolates, particularly those living alone in one room without any meaningful social ties (18, 23). A clue as to the reason for these discrepant findings comes from some of the recent formulations of the pioneer microbiologist Rene DuBos. DuBos maintains that

> "The sciences concerned with microbial diseases have developed almost exclusively from the study of acute or semi-acute infections caused by virulent microorganisms acquired through exposure to an exogenous source of infection. In contrast, the microbial diseases most common in our communities today arise from the activities of microorganisms that are ubiquitous in the environment, persist in the body without causing obvious harm under ordinary circumstances, and exert pathological effects only when the infected person is under conditions of physiological stress. In such a type of microbial disease the event of infection is of less importance than the hidden manifestation of the smoldering infectious process and than the physiological disturbances that convert latent infection into overt systems and pathology" (39).

According to DuBos, microbial disease is not necessarily acquired through exposure to a new microorganism. In a large number of cases clinical manifestations of disease can occur through factors which disturb the balance between the existing ubiquitous organisms and the host that is harboring them. Under conditions of crowding this balance may be disturbed, but this disturbance would not be simply a function of the physical crowding (that is, the closeness of contact of susceptibles to carriers of the microorganisms) but of other processes.

In a search for those environmental factors which may affect these homeostatic mechanisms of the host and profoundly influence his susceptibility to disease, attention has recently refocused on a set of factors which, though difficult to quantify, may be of crucial importance, namely *the presence of other members of the same species.* Viewed in this context, the importance of other members of the same species lies not so much in the microorganisms they harbor as in the pattern of relationship that exists among the members.

Some of the more convincing evidence supporting the point of view that such social processes can influence health comes from animal studies. To a large extent, these have been concerned with variations in the size of the group in which the animals interact and in situations which lead to confusion over territorial control. A number of investigators have shown, for example, that as the number of animals housed together increases, with all other factors such as genetic stock, diet, temperature, and sanitation kept constant, maternal and infant mortality rates rise, the incidence of arteriosclerosis increases, resistance to a wide variety of insults, including drugs, microorganisms, and x-rays decreases and there is an increased susceptibility to various types of neoplasia (40–48). Lack of territorial control has been shown to lead to the development of marked and persistent hypertension in mice, to increased maternal and infant mortality rates and also to reduced resistance to bacterial infections and decreased longevity (49).

In addition to demonstrating the health effects that follow these variations in the social milieu, further animal studies have provided clues as to the processes through which they may be produced. Changes in group membership and the quality of group relationships have been shown to be accompanied by neuroendocrine changes, particularly, but not exclusively, by changes in the pituitary and adrenal-cortical systems (50–51). The changes in some of these hormones such as the 17-hydroxycorticosteroids and the catecholamines, especially if prolonged, can, by markedly altering the homeostatic mechanisms of the body, decrease resistance to a wide variety of insults. Thus, the phenomenon has been well documented in a number of animal species by studies which are methodologically sound and reasonable biologic explanations for the findings.

Convincing as this animal work would appear to be, the relevance to human health is as yet unproved, and considerable doubt exists as to the appropriate analogues in the human social system. As indicated above, attempts to demonstrate that increased population density and crowding are related to poorer health status in humans have been unconvincing and have led to confusing and often conflicting results. A careful review of some of these studies taken in conjunction with the animal work suggest that, for future research in this area to be profitable, we should abandon a search for the direct human counterpart to animal crowding or territorial confusion and concentrate instead on some more general principles or hypotheses that can be derived from these data. In my view, four such principles seem worth considering.

The first of these can perhaps best be stated as an hypothesis. This would hold that a crucial process linking high population density to enhanced susceptibility to disease is not due to crowding per se, but the result of disordered relationships that, in animals, are inevitable consequences of such crowding. These, while being manifest by a wide variety of bizarre and unusual behaviors, often have in common a failure to elicit anticipated responses to what were previously appropriate cues, and an increasing disregard of traditional obligations and rights. Thus, habitual acts of aggression (including "ritualized" aggression) or subordination on the part of one animal fail to elicit appropriate reciprocal responses on the part of another. Characteristic obligations and responsibilities become blurred, for example, female rats cease caring for their young and male-female relationships become disturbed to a point where the equivalent of "gang rapes" have been reported in rats under conditions of high population density. This failure of various forms of behavior to accomplish the intended results (that is, to lead to predictable responses on the part of others) leads to one of three types of responses on the part of the animals involved, the most common of which is a repetition of the behavioral acts. Such acts are, of course, always accompanied by profound neuroendocrine changes, and presumably their chronic repetition leads eventually to the permanent alterations in the level of the hormones and to the degree of autonomic nervous system arousal found under conditions of animal crowding. The fact that these behavioral acts are in a sense inappropriate, in that they do not modify the situation, can be expected to enhance such hormonal changes. Under these conditions it is not difficult to envisage the reasons for the increased susceptibility to environmental insults displayed by such animals.

An alternative response on the part of some animals is to withdraw from the field, to remain motionless and isolated for long hours on end. It is apparently not uncommon, for example, to observe some mice under crowded conditions crouched in most unusual places—on top of the razor-thin edge of a partition or in the bright light in the center of the enclosure—completely immobile and not interacting with any other animals. Such animals apparently do not exhibit the increased pathology demonstrated by the interacting members (40).

The third alternative is for animals to form their own deviant groups, groups that apparently ignore the mores and codes of behavior of the larger group. Thus, "gangs" of young male rats have been observed invading nests, attacking females, and otherwise behaving deviantly. I am not aware of any data on the health status of these gang members, but according to this hypothesis they also should not exhibit any increase in pathology.

This hypothesis suggests that, in human populations, increased susceptibility to disease should occur when, for a variety of reasons, individuals do not receive any evidence (feedback) that their actions are leading to desirable and anticipated consequences (that is, in situations analogous to the first category of animal responses). In particular, this would be true when these actions are designed to modify the individual's relationships to the important social groups with whom he interacts. Such circumstances might occur in a variety of situations. First, it is highly probable that when individuals are unfamiliar with the cues and expectations of the society in which they live (as in the case of migrants to a new situation, or those individuals involved in a rapid change of social scene, such as the elderly in an ethnic enclave caught up in urban renewal), their actions would be unlikely to lead to the consequence they anticipate and thus, due to the chain of events suggested above, they should be more susceptible to disease than are those for whom the situation is familiar.

Some circumstantial evidence supporting this point of view exists. Scotch (52, 53) found that blood pressure levels among the Zulu who had recently migrated to a large urban center were higher both than those who had remained in their rural tribal surroundings and those who had lived for over ten years in the urban setting. In two independent studies Syme (54–56) has demonstrated that occupationally and residentially mobile people have a higher prevalence of coronary heart disease than stable populations, and that those individuals displaying the least continuity between childhood and adult situations, as measured by occupation and place of residence, have higher rates of coronary heart disease than those in which more continuity could be determined. Tyroler and Cassel designed a study in which death rates from coronary heart disease, and from all heart disease, could be measured in groups who were themselves stable but around whom the social situation was changing in varying degree. For this purpose they selected 45- to 54-year-old, white, male, rural residents in various counties of North Carolina and classified those counties by the degree of urbanization occurring in that locality. Death rates for coronary heart disease and all heart disease showed a stepwise increasing gradient with each increase in the index of urbanization of the county (57). In a further study, Cassel and Tyroler (58) examined two groups of rural mountaineers working in a factory. The first of these was composed of individuals who were the first of their family to engage in industrial work, while the second comprised workers who were the children of previous workers in this factory. The two groups were drawn from the same mountain coves and doing the same work for the same pay. The underlying hypothesis was that the second group, by virtue of their previous experience, would be better prepared for the expectations and demands of industrial living than the first and would thus exhibit fewer signs of ill health. Health status was measured by responses to the Cornell Medical Index and by various indices of sick absenteeism. As predicted, the first group had higher Cornell Medical Index scores (more symptoms) and higher rates of sick absenteeism after the initial few years of service at each age.

A second set of circumstances in which this lack of feedback might occur would be under conditions of social disorganization. While still being far from a precise term which can be measured accurately, social disorganization has proved to be a useful concept in a number of studies. In the hands of several investigators, for example, various indicators of social or familial disorganization have been related to increased rates of tuberculosis (23), mental disorders (59), deaths from stroke (60), prevalence of hypertension (61), and coronary heart disease (54). Clearly, more work needs to be done in clarifying and quantifying this concept, but until there is recognition of what needs to be clarified or quantified, little progress can be anticipated.

A second general principle which emerges from the animal work is that not all members of a population are equally susceptible to the effects of these social processes. Systematic and regular differences have been observed with the more dominant animals showing the least effects and the subordinate ones having the most extreme responses (62). Those differences are manifest both in the magnitude of the endocrine changes and in increased morbidity and mortality rates. Conceivably these findings may, in part, explain the high levels of blood pressure found in American blacks who not only usually occupy a subordinate position in society, but whose lives are frequently characterized by considerable evidence of social and familial disorganization.

A third principle is concerned with the available protective factors—those devices which "buffer" or "cushion" the individual from the physiologic or psychological consequences of these social processes. These would seem to be of two general categories: biologic and social. The biologic category would include the adaptive capacities of all living organisms—the capacity, given time, to adjust physiologically and psychologically to a wide variety of environmental circumstances. In animals, this is illustrated by the higher responses of laboratory-naive animals to given stimuli than of veteran animals (63), and to the much lower rate of pathology in animals born and reared in crowded conditions than in animals transferred to these conditions some time after birth (64). In humans, the finding that death rates from lung cancer in the United States, when controlled for cigarette smoking, are considerably higher in the farm-born who migrated to cities than in lifetime urban dwellers (despite the longer exposure of the latter to atmospheric pollution) (65), would seem to be evidence of the same phenomenon.

In addition to these biologic adaptive processes, various social processes have also been shown to be protective. Chief amongst these are the nature and strength of the group supports provided to the individual. In rats, for example, the efficacy with which an unanticipated series of electric shocks (given to animals previously conditioned to avoid them) can produce peptic ulcers is determined, to a large extent, by whether the animals are shocked in isolation (high ulcer rates) or in the presence of litter mates (low ulcer rates) (66). The territorial conflict which led to elevated blood pressures quoted above (49) was produced by placing mice in intercommunicating boxes. Hypertension only occurred, however, when the mice were "strangers." Populating the system with litter mates produced none of these effects. In humans, small group studies have shown that the degree of autonomic arousal (produced by requiring solutions to what in reality are insoluble tasks) is more extreme if the group is made up of strangers than when it is made up of friends (67). As reported above, modern studies on the epidemiology of tuberculosis in the United States and Britain have shown that the disease occurs more frequently in "marginal" people, that is, those people who are deprived of meaningful social contacts (18, 23).

If these three principles, or hypotheses, are correct, it would appear that health changes which are dependent upon the presence of other members of the same species will not be universal—not affecting all people in the same manner. A more adequate formulation would hold that such consequences will be dependent on:

A) The importance or salience of the relationships that become disordered.

B) The position of the individuals experiencing such disordered relationships in the status hierarchy.

C) The degree to which the population under study has been unprepared by previous experience for this particular situation (that is, has had insufficient time to adapt).

D) The nature and strength of the available group supports.

The final general principle that can be derived from the animal experiments relates to the manifestations of ill health that might be anticipated under conditions of social change and disorganization. The model of disease causation provided by the germ theory has accustomed us to think in monoetiologic specific terms. Accordingly, much of the work concerned with social or psychological antecedents to disease has attempted to identify a particular situational set (usually labeled "stress" or "a stressor") which would have a specific causal relationship to some clinical entity analogous, say, to the relationship between the typhoid bacillus and typhoid fever. Such a formulation would appear to be clearly at variance with the animal data, a striking feature of which is the wide variety of pathologic conditions that emerge following changes in the social milieu. A conclusion more in accordance with the known evidence would be that such variations in group relationships, rather than having a specific etiologic role, would enhance susceptibility to disease in general. The specific manifestations of disease would be a function of the genetic predisposition of the individuals and the nature of the physico-chemical or microbiologic insults they encounter. This concept of generalized susceptibility is consistent with the situation in the United States where it has recently been demonstrated that those regions of the country having the highest death rates from cardiovascular disease (age, race, sex specific) also have higher than expected death rates from all causes, including cancer and infectious diseases (68). This illustration, of course, does not necessarily document that social processes are responsible for such an increased susceptibility, but does lend credence to the view that variations in generalized susceptibility may be a useful concept. Somewhat more direct evidence is provided by Christenson and Hinkle in an industrial study in the United States in which they have shown that company managers who, by virtue of their family background and educational experience, were least well prepared for the demands and expectations of executive industrial life, had the highest rates of all diseases—major as well as minor; physical as well as mental; long-term as well as short-term (69). In the studies on tuberculosis referred to above (23), the highest rates of disease were found in "marginal" people, that is, people who, for a variety of reasons (ethnic minorities rejected by the dominant majority, people with high sustained rates of mobility or broken family life), were deprived of meaningful social contact. A similar set of circumstances has been observed in the case of schizophrenia, multiple accidents, suicide, alcoholism, and other respiratory diseases (70–74). While the manifestations of ill health under these circumstances are quite diverse, the individuals share in common an increased susceptibility to disease.

Presumably the causes of disease may vary under different conditions. In preindustrial societies where people live in small, tightly organized communities, the exposure to highly potent disease agents may account for the major part of disease causation. Under these circumstances, variations in susceptibility due to social processes may be of relatively little importance. With increasing urbanization, populations become increasingly protected from such disease agents, but simultaneously, they are exposed to the social processes discussed above. Variations in susceptibility now assume greater importance in the etiologic picture and the concomitant changes in such factors as diet, physical activity, and cigarette smoking will facilitate the emergence of new manifestations of such susceptibility.

This formulation would suggest that preventive action in the future should focus more directly on attempts at modifying these psychosocial factors, on improving and strengthening social supports, and reducing the circumstances which produce ambiguities between actions and their consequences. While these are not easy ideas to make operational, I do not believe they are beyond the bounds of human ingenuity, once the factors specific to any particular situation have been made explicit. Such research and the intervention strategies that would flow from it might indeed bring us closer to the vision James had and make our actions more consonant with our rhetoric.

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# Behavioral Sciences and Health

ALEXANDER H. LEIGHTON, M.D. AND JANE M. MURPHY, PH.D.

Dr. James in one of his articles says "Human behavior is strongly implicated in our first four leading causes of death: heart disease, cancer, stroke and accidents. These . . . are responsible for nearly three times as many deaths as all other conditions put together" (1).

In this passage, Dr. James is using death in order to make a point forcefully about human behavior. Elsewhere in his writings he emphasizes the waste and misery from chronic morbidity that is attributable to these same disorders, and to others such as arthritis. In this context it would seem appropriate for one also to add mental illness.

The recognition of human behavior as a major component in the complex of causes leading to these diseases means that the medical profession is finding itself today in a much larger arena than ever before in history. Prevention of illness and prevention of relapse are dependent upon concerted action by the medical profession in association with numerous other groups seeking to reduce and control the stresses that arise from the social conflicts, population pressures, and economic difficulties characteristic of our time. Government, education, business, unions, consumer groups, minorities, mass media, religion, and the world of arts and letters are all involved, together with such disciplines as political science, economics, public and business administration, engineering, architecture, sociology, anthropology, and psychology. Further, it is not only in the area of preventing disease that human behavior has an impact on the health field. Its influence is equally strong in areas of policy development and implementation and in the management of services. Health, then, is clearly not the exclusive product of medical treatment and the patient's own behavior; it relates also to the behavior of planners, caretakers, and legislators.

A scope of this magnitude presents serious problems for the physician and nurse. On the one hand, they cannot become expert in all the fields to which the logic of delivering service and preventing disease leads. On the other hand, it does not seem right or wise to abandon everything to the political arts. Two major possibilities are evident: 1) enlarging the categories of health professionals through training a variety of specialists who can form a bridge between the biomedical and behavioral sciences; 2) making available some training in the social and behavioral sciences to every physician and nurse, both as part of their original professional education and as a component in continuing education. What follows is addressed mainly to the topics under 2).

"Behavioral Sciences" is not yet a standard term. In the broadest sense, it

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covers a range from biological methods of studying behavior such as experiments on brain function, to disciplines concerned with group phenomena such as economics and political science. A more restricted usage, and the one we shall employ here, gives main emphasis to psychiatry, psychology, social psychology, sociology and cultural anthropology. The current popularity of the label is probably due to the fact that the Ford Foundation at its inception had a Division of Behavioral Sciences. Berelson, formerly head of this Division, can be consulted for a more extended discussion (2).

Our concern is the possible contribution of these sciences toward improving health. This suggests the need for a conceptual framework that will help in grasping the complexity of factors that impinge upon health and illness. In biology it has long been the custom to speak of different "levels of integration," for example, the chemical, the physiological and the functioning of the total organism. The physiological level includes phenomena that are not entirely predictable on the basis of what is known about chemical properties. It is, in short, not merely the summation of chemical attributes but rather the product of their integration in particular ways so as to create phenomena not otherwise seen, and certainly not in chemicals each considered separately. Similarly, the functioning of the total organism is more than the sum of the physiological components. It is their integration in such a way as to produce a "higher" level of phenomena, namely, an individual.

There are, of course, more levels than the three mentioned above, but they can suffice for pointing out that each constitutes a field of study. Any given level is dependent on those "below" and each contributes to those "above," yet at the same time each has its own idiosyncratic theories; each is a scientific area in its own right.

A useful analogue to levels of integration can be laid out in regard to the behavioral sciences. Here the behavior of the individual human organism is the starting point, or "lowest" level and the behavior of the community is the "highest." This scheme is presented in the left-hand column of Table I, organized in terms of four levels. A fifth level could be postulated representing world society, since at the present time virtually all members of the human species are indirectly connected and so affect each other, interdependent like the winds and currents of the globe. For the purposes of this article, however, the four are probably sufficient. The point to make is that each, except the first, refers to commonly occurring human groupings and all involve behavior and opportunities for influencing behavior with regard to health. It is also to be noted that each level has its own properties and phenomena and that the theories and methods of understanding and inducing change are different.

The middle column indicates some of the behavioral science disciplines (in our restricted usage of the term) that are relevant to the levels. As can be seen, there is not a one-to-one relationship. This is because each of the disciplines covers a large area and has some degree of overlapping with its neighbor. It is apposite also to observe that not all parts of all the behavioral science disciplines are relevant to the problems of modifying behavior to improve health.

TABLE I

*Selected Behavioral Sciences and the Change Process**

| Level | Main relevant disciplines | Some techniques for managing and changing behavior |
|---|---|---|
| 4. Communities (villages, towns, cities) | Sociology<br>Anthropology | Political action<br>Management techniques<br>Applied sociology<br>Applied anthropology |
| 3. Bureaucracy (government, business, private agencies, etc.) | Sociology<br>Anthropology<br>Human relations<br>Social psychology | Management techniques<br>Confrontation and negotiation<br>Training programs<br>Applied sociology<br>Applied anthropology |
| 2. Small groups (committees, boards, special interest groups, etc.) | Social psychology<br>Psychology<br>Psychiatry | Parliamentary procedures<br>Group dynamics<br>Group psychotherapy<br>Encounter groups |
| 1. Individual | Psychology<br>Psychiatry | Presenting facts, argument and persuasion (Socratic and dialectic)<br>Reward and punishment<br>Psychiatric treatment<br>Brainwashing<br>Hypnosis<br>Chemotherapy<br>Lobotomy |

* The development of this chart has benefited from discussion with Robert C. Benfari, Ph.D. and Douglas Porter, Ed.D.

Anthropology, for example, includes archeology, physical anthropology, linguistics as well as cultural anthropology. Of these, cultural anthropology is probably the most pertinent and archeology the least.

In the right-hand column of Table I are listed various techniques for changing behavior. The categorization here is still more rough but it holds together in terms of a first approximation. As can be seen, these techniques are only in part based on scientific investigation; they include also various practices and ideas derived from religion, philosophy, political ideology, folklore, and craftiness handed down through generations by those engaged in influencing their fellow humans.

Starting with the individual at level one, it can be said that the conceptual framework for changing behavior lies in theories of personality, generated mainly by the disciplines of psychiatry and psychology. These theories, together with practical experience, have led to the conclusion that effecting fundamental change in personality (chemical and surgical methods aside) is exceedingly difficult. When change can be accomplished, it requires much time, professional effort, expense, and very often high motivation on the part of the individual to be changed.

Despite this, many people are apt to think almost exclusively in terms of individuals when they consider the problem of changing behavior. This is perhaps especially true of physicians. Since the quick methods of drug treatment and surgery are neither ethical nor very controllable outside of certain limited therapeutic situations, it is small wonder that an air of pessimism is common, and folk dicta such as "you can't change human nature" prevail. While methods based on psychotherapeutic procedures of various kinds can help some people stop smoking, eat more reasonably and adopt other behaviors conducive to health, general success, especially lasting success, is far from noteworthy (3).

Fortunately the outlook appears to be better on some of the other levels, and especially if effort is made at more than one level simultaneously. It is important, however, not to swing from exclusive focus on the individual to complete neglect. The understanding of personality process and the kinds of things that affect it has a basic relationship to understanding the interpersonal processes that go on at the other levels of integration and hence the topic of personality, including the development and functioning of "healthy" personalities, is appropriate in medical education, over and above its essential place in psychiatry.

As an entry into the next level, small groups, let us consider first the growing evidence that some people are able to change their behavior more radically and more permanently in a group context than by isolated effort. The effectiveness of Alcoholics Anonymous is a case in point, as are also the more recent weight control groups like Weight Watchers and TOPS. It is our impression that in the future more and more physicians will be recommending that their patients join such groups. We may be near a time when a doctor will be as likely to prescribe such group involvement as he is to prescribe medication or recommend psychotherapy. Given the goals of medical training as they have existed it is reasonable to say that the physician knows the general mechanism by which a particular drug functions when he prescribes it and he knows generally what kind of treatment a patient will receive if he makes a referral to a conventional psychiatrist. It is less likely, as things stand now, that he will know how a small group operates, what processes lead to behavior modification, and what possible weaknesses deserve alertness.

To shift attention from individual to group is to cross a major divide. This is because such conceptualization means thinking of the group as an open system, or quasi-organism, which has its own properties and action tendencies and a measure of independence from the personalities which happen to compose it at any given time. The step is intellectually difficult, especially for those clinicians who have always thought primarily in terms of individuals, and who consequently tend to regard groups as a kind of sum of individual components and hence infinitely variable and unpredictable.

Small groups have been extensively studied, often under experimental conditions and a good deal is known about their characteristics (4–8). Thus, the nature of the tasks to be performed affects the group's structure and patterns of functioning. For example, interaction in the group is quite different if the job is: 1) to reach a decision by voting, 2) to create a slate of suggestions by general consensus, 3) to learn how to do something by listening to an outside expert, or 4) to conduct a joint task such as getting out a mailed brochure. It is also clear that a group develops additional "tasks" pertinent to its own maintenance such as sociability, a sense of belonging, or mutual support. These may at times take priority over the original designated task.

Considerable action is carried out by a group in the early phase of its existence whereby roles are defined and linked together in a dynamic equilibrium so that conflict within the group is controlled, the individual motivations are channeled toward group goals, and information-gathering and decision-making procedures are established. As part of this, a balance is struck between the designated or "primary" task of the group and the additional tasks that have to do with maintaining social and emotional stability. So long as the two kinds of tasks involve compatible goals, they have a reinforcing effect and it is appropriate to speak of high morale. From time to time, however, the designated task calls for action that is not compatible with social and emotional tasks, and in such a situation it is common for a small group to make compromises that greatly reduce effectiveness with regard to the primary task. Indeed the social and emotional features may at times take precedence over everything else. When this happens in a small group with a designated health task, the potential for achievement can be totally vitiated.

To illustrate some of these points about group process, let us take the case of a board of directors of a health agency. The agency was formed to provide the services of a medical specialty and the board was charged with the task of maintaining an adequate service, and had the authority to hire and fire the agency staff. There was medical representation on the board, but most of the members were laymen who had been elected by a process intended to bring about representation of the interests of the clients and the population from which they came. The board members met periodically around a table and conducted their business through discussion and voting.

After several years an issue arose regarding one of the agency's professional staff. Information came through certain board members and from individuals in the community which suggested that the staff members' work was in serious difficulties. Although there was much discussion at board meetings and among individual members at other times, the board did not make any formal move to: 1) ascertain the facts or 2) work with the agency staff to clear up the difficulties. The situation continued for over four years during which time the agency's services declined to the point where many physicians in the community had stopped referring their patients and other service agencies had stopped sending their clients. What had been an active and forward-looking agency became what one of the general practitioners described as "a shell."

How could a board formed to represent the clients allow such a thing to happen? Most of the analyses that went on in the board, in the community and in the relevant branches of the town and state government were to the effect that the trouble was an unfortunate concatenation of personalities and circumstances. Yet this kind of paralysis is a frequent occurrence in small groups, and can perhaps more wisely be attributed to malfunctioning of the group process than purely in terms of personality clashes. The social and emotional needs for avoiding conflict seemed gradually to have clouded over the primary task of providing adequate service to patients.

The patterns of functioning characteristic of groups both set limits to, and create opportunities for, the members, if they have some knowledge of the processes to be expected. The importance of this is evident when one reflects on the part played in our society by small groups like boards, advisory councils, and executive committees. These are often focal points for exerting considerable influence toward changing human behavior, and in the health field are frequently the locus for policy formation and implementation. Since health professionals may be members of such groups or have access to them it would seem highly desirable that they have formal training in this branch of social psychology. Such training has long been available to and commonly utilized by those concerned with the management of human affairs in business, industry, government, the military, and even in religious groups, but so far as we are able to determine it has been comparatively little utilized in the field of medicine.

The next line upward on the chart refers to bureaucratic organizations, each of which can be regarded as a hierarchical network that contains within it numerous small groups, both formal and informal. These organizations constitute large social systems concerned with economic, governmental and other tasks relevant to the functioning of society.

Bureaucracies have been a focus for study, speculation and theory-building since long before the emergence of the behavioral science disciplines as we know them today. It is probably safe to assume that bureaucracies have been a topic for discussion ever since humanity began to gather in cities and face problems of social management and division of labor. During the last two hundred years economists, political scientists, sociologists, and more recently anthropologists have been major contributors toward understanding the nature of this particular type of social organization (9–11). Formerly the emphasis was mainly analytic, but there has been a turn toward empiric methods based on data collection and systems analysis. The methods include stationing observers to record transactions at key points in a bureaucratic organization and the use of questionnaire surveys. Time measurements and quantitative data are thus obtained, and the understanding of change processes greatly enhanced. In essence these techniques concern the study of social phenomena in natural settings and thus stand in some contrast to the laboratory-like experimental approach that can be applied to small groups. One can point to a parallel here in the difference between clinical and basic medical research. Although crude, the parallel is not without point. Experimentation is possible in bureaucratic social systems just as it is in clinical work, but circumstances generally require much greater dependence on observation and on taking advantage of "experiments of nature."

Psychology has also played a part in studies of functioning in large organizations, particularly with reference to roles and role stresses on individuals. The term "human relations" has been employed to refer collectively to work in this context that is derived from the behavioral sciences (12–15). The theories and viewpoints of human relations research at times run counter to explanatory systems based on economic motivation and have done much to illuminate why organizations on occasion do things that are off target so far as their designated tasks are concerned and seemingly contrary to what common sense would suggest as economic self-interest.

While bureaucracies are at a level different from small groups, they nevertheless share characteristics common to all social systems. Among these are the processes of both internal and external equilibrium. The internal has to do with maintaining the organization's existence through the control of conflict among its parts, the channeling of information, the distribution of rewards and punishments, and the indoctrination of new members. The external is concerned with holding or improving the organization's place in relation to the other systems with which it must interact. In bureaucracies these interrelated functions are enormously complex and are no more than partially understood by the participants, including the holders of highest office. It is only through modern studies that a beginning is being made toward grasping the totality.

The relevance of all this to medicine lies first in the fact that hospitals, whether public or private, are bureaucracies. Most other health agencies have bureaucratic features as do schools of medicine, dentistry and nursing, and the medical societies. It would seem appropriate, consequently, for health professionals to be well informed regarding the processes characteristic of these kinds of social systems and to be in as favorable a position as possible for enhancing their potentialities and minimizing their liabilities—particularly the tendency to get so involved in social and emotional maintenance activities and agency survival as to go more or less off target with regard to promoting health. Numbers of times it has been observed that the patient in the hospital bureaucracy experiences situations which heighten anxiety and apprehension when these most need to be reduced. This is often because the main therapeutic goal becomes overshadowed by other goals related to the equilibrium-maintaining functions of the system. Such effects give rise to the saying that "hospitals are run for doctors and nurses, not for patients." It seems highly probable that a significant part of the criticism leveled today at health services is due to the working of maintenance processes in the relevant bureaucracies, processes that are poorly understood and hence have been allowed to become malfunctional with regard to the primary goals—that is, treating the sick.

There is no reason to believe that health systems are intrinsically worse than other bureaucracies, but rather that like many others they are in major need of understanding, evaluation, testing, and overhaul. This point is underscored when it is realized that there have been a number of behavioral science studies of health systems (16, 17). Unfortunately, it appears that the results have not become widely known among health professionals and one cannot escape the impression that utilization of concepts, theories, and research findings has been greater in a number of business organizations, such as the telephone company.

Since our concern in this article is with promoting health through changing behavior, the scope of interest is wider than medical agencies as such. One of the routes whereby health professionals can seek to alter unhealthy patterns of behavior is through interaction with nonmedical agencies that have a potential for wide influence directly on their members and indirectly on others. Welfare and educational institutions, industrial enterprises, and unions are some examples.

Turning now to the top level of the chart, the notion of community that we are employing here can be illustrated by pointing to a rural town. It is again a social system, but this time one that consists of an integration of numerous groups. In addition it occupies a circumscribed area and, unlike small groups or bureaucracies, is a system in which individuals can spend all the hours of the day and all the days of their lives. Further, it has greater permanency or durability and is comparatively self-sufficient. Where a bureaucracy is like a part or an organ, a town is like an organism.

The word "integration" implies what sociologists call structure, namely regular, repeated patterns of behavior through which the town's functioning is accomplished. These include a number of patterns not hitherto mentioned. One is the family—both the nuclear unit of parents and children, and the extended kinship network. Another is stratification in terms of socioeconomic classes which reflect division of labor, prestige, and influence. There are also numerous informal associations of persons which are neither small groups, as the term has been used here, nor bureaucracies. In many instances there are important divisions into minorities.

The orchestration of the whole is made possible through patterns of shared sentiments or reciprocal values and opinions which make up a considerable part of what is meant when the word "culture" is used. Cultural differences among communities may be described as different styles whereby the "functional prerequisites" of a social system are achieved. These prerequisites include such matters as the acquisition of food, clothing and shelter, protection against dangers, the promotion of health, family formation, and the indoctrination of children into the ways of the community—those essential patterns, in short, which are necessary for the survival of the community (18, 19).

Having posited the town as a model in order to explain the concept of a community, it is necessary now to introduce qualifications. The principle one is to point to the obvious fact that a large part of humanity lives in huge urban and suburban aggregates that appear far different from the rural town. The difference, however, may be regarded as one of degree and variation that nonetheless invokes the application of behavioral science concepts and methods. The small town model of a community can be held as a possibly useful anchor point of reference.

Integration, culture, and structural change are key ideas for approaching the problem of modifying human behavior in a more healthy direction. One may regard culture as a kind of computer program carried in the heads of the people whereby the integration of the social system is achieved and maintained. The resultant equilibrium, however, is dynamic; that is to say, change is constantly in progress. Events are forever occurring within the community to which adjustments have to be made, or arising outside and impinging upon it with parallel consequences. The result is that communities alter throughout time. Integration and culture put brakes on very rapid alteration, but because they are also geared to adaptation they play a part in the emergence of major change in the course of years. This can be clearly seen if one compares the eighteenth century with today in such matters as public attitudes and behavior in regard to sanitation, treatment of the poor, working children, slaves, and public executions. This is not to imply that the changes have been either fast enough or extensive enough, but it does counter the notion that human behavior never changes or never changes in a direction most of us would consider better.

The fact that there is some movement in social systems opens the way for altering behavior in a direction conducive to improved health. One approach, derived from anthropology for the most part, can be summed up as "enhancing movement that is already there" or, in short, acting as a catalyst. An essential preliminary for bringing this about is an adequate diagnosis of the social processes and felt needs present in the community. An illustration may help to clarify the point.

Some years ago, the late Allan Holmberg, Professor of Anthropology at Cornell, undertook what he called "participant intervention." Cornell, in collaboration with the Indigenous Institute of Peru, arranged to rent Vicos, "a publicly owned hacienda on which previous observational studies had been made, for an initial period of five years. Broadly speaking, the purpose of embarking on this experience was two-fold: on the theoretical side, it was hoped to conduct some form of experimental research on the processes of modernization now on the march in so many parts of the world; on the practical side, it was hoped to assist the community to shift for itself from a position of relative dependence and submission in a highly restricted and provincial world to a position of relative independence and freedom within the larger framework of Peruvian national life" (20).

The Vicos hacienda is surrounded by "snow-capped peaks of the Andes, some of them over 20,000 feet in height. It contains a land area of from 30,000 to 40,000 acres, about 2,500 of which are now under cultivation and another 4,000 of which are utilized for grazing purposes. It is very rocky and hilly, rising in elevation from about 9,000 to 14,000 feet. The lower parts of the hacienda are used largely for agricultural purposes, the upper parts, almost exclusively for grazing.

"Like many properties of the Andes, Vicos belongs to the state. Formerly it was leased for exploitation to the highest bidder at public auction every 10 years. Attached to the land but owning none of it are 380 families of Quechua-speaking Indians, 2,250 people, most of whom live on the lower part of the hacienda on scattered farmsteads ranging from one to 15 acres in size, occupying roughly 90 percent of the arable land. The remaining ten percent of the land was previously farmed for commercial purposes by the lessee of the hacienda, with Indian labor supplied without charge—by custom of long standing, one adult member of every household was obligated to pay a labor tax of three days a week for the right to occupy a small plot of land for subsistence purposes."

Long-range goals involved improvements in economic resources, education and health, but part and parcel of this was the aim of "developing independent problem-solving and decision-making organizations among the indigenous population which could gradually assume the control and direction of community affairs in a rational and humane manner. To start these processes in action, a great many specific steps were designed, all of which emerged from a thorough base-line study of the community and the surrounding area."

Two examples of Holmberg's "specific steps" will serve to illustrate the general approach. An ancient custom of *mando* involved a weekly meeting during which the hacienda foreman assigned work tasks to the labor force for the ensuing week. Gradually the meetings changed into discussion sessions. The members of the labor force were asked for their views regarding work alternatives, and then with regard to new ideas and proposals. At the same time the Indian *mayorales* or strawbosses who had been designated by the previous proprietor of the hacienda also had their meetings (with the foreman) converted into discussion sessions. In effect, they became a small group capable of making collective decisions. Thus the *mando* became a vehicle for education and the *mayorales* became an executive committee for running the hacienda. The devolution of power was completed when the *mayorales* became a governing council elected annually by the community and the original individuals were gradually replaced by younger, better educated men with progressive ideas. The final step, achieved after some considerable political resistance from other hacienda proprietors, was that of turning the ownership of the Vicos hacienda over to the Indians and the withdrawing of Cornell (21).

Thus, in the context of a community, of an antiquated bureaucracy, and of small group meetings, changes were catalyzed that had far-reaching effects. Aside from reorganizing the community's economic pattern and giving each family more wealth and resources than they had previously known, there was also the development of schools and medical services and marked improvement in behavior relative to health practices, especially with regard to the care of children. More food and available money did much toward making this possible.

We have chosen the Vicos case because it illustrates working at all four of the levels shown on the chart, because the outcome was successful in bringing about profound changes in the behavior of many people in the space of a few years; and because, among the changes, patterns relevant to improved diet, baby care, and other practices important in health were prominent.

In concluding, we would like to come back to what was said earlier about the difficulty of changing individuals. Although it may seem contrary to the expected, it is often easier to bring about change in communities than in individuals, and easier to introduce health changes in a larger package of community development than by focusing on health alone (22) (23).

In the main, however, experience suggests and theory supports the idea that changes in behavior are more likely to come about if effort is made simultaneously at all four of the levels shown on the chart. To make this effective, however, health professionals need a grasp of social processes and some practice in working with other professionals and with client groups in congruent planning and congruent action.

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# George James and the New Nutrition: Community Medicine as a Spearhead of Comprehensive Health Services

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George James was *the* pioneer health administrator to fully appreciate the significance of contemporary community nutrition to the public's health. While in the New York State Department of Health, he helped organize the Albany Cardiovascular Health Center which explored the importance of serum lipids in coronary heart disease (1). Dr. James, as First Deputy Commissioner of Health for the City of New York, and Commissioner Leona Baumgartner exhibited verve in using public funds to support the Anti-Coronary Club which was formulated by Dr. Norman Jolliffe and his colleagues of the Bureau of Nutrition. This was the first prospective epidemiologic program designed to test the hypothesis that free-living subjects could adhere to a cholesterol-lowering regimen (the "Prudent Diet"), and that this nutritional program could have a favorable effect on coronary heart disease morbidity and mortality (2).

By fluoridating New York City's water supply, George James' concept of a "managerial" approach to public health nutrition was implemented. He also gave great impetus and support to the Bureau of Nutrition under the author's directorship following the death of Dr. Jolliffe in 1961. The Anti-Coronary Club and the Bureau of Nutrition's clinic and research programs were expanded under his aegis (3). Adipose tissue aspiration was conducted for the first time in an epidemiologic experiment as an objective monitor of linoleic acid intake; the Prudent Diet was assessed for nutritional adequacy by multiple serum vitamin determinations; vanil mandelic acid studies were conducted to metabolically study psychic stress factors in the Anti-Coronary Club experimental and control groups. These are examples of how nutrition research could flourish in a progressive health department setting (4).

Following the study of Stare in 1945, a comprehensive nutrition survey was not conducted in New York City until 1965, when George James was Commissioner of Health (5). This detailed nutritional evaluation of 642 children of the lower East Side of Manhattan, in addition to the more classical methods of assessing nutritional status, utilized microbiologic assay methods of twelve serum vitamins, amino acid ratio, serum cholesterol, triglyceride, and adipose tissue fatty acid analysis. These studies, along with anemia surveys in the Bedford-Stuyvesant area of Brooklyn, provided the first clue that clinical and subclinical malnutrition might be a problem in New York City school children.

George James emphasized that the critical mass of malnutrition and poverty is a powerful destructive force for individuals and society (6). In developing countries, nutritional deficiencies are recognized as playing a major role in

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initiating and maintaining the vicious cycle of malnutrition, disease, chronic fatigue, illiteracy, low productivity, poverty, inability to attain social and economic independence, and back again to another cycle of a malnourished and impoverished generation. The corollary of this for Inner City, USA might be: suboptimal malnutrition in utero; suboptimal malnutrition in infancy; pyschosocial maldevelopment in the inner city; exposure to suboptimal motivational, cognitive, and affective responses to life; inability to attain satisfactory grades; inability to compete academically or economically; psychosocial depression; asocial behavior, including drugs and crime; and inability to optimally exercise citizen rights and responsibilities to the maximum of genetic potential (7), with reproduction of a second-generation cycle with similar attributes.

Social development and nutritional well-being go hand in hand with civilization; it is, in fact, difficult to separate the two or define the specific role the correction of malnutrition has on social advancement. In New York City from 1900 to 1930, the infant mortality rate from respiratory and gastrointestinal infections fell from 75/1000 to 17/1000. This occurred at a time when vaccines and antibiotics were not to be discovered until decades later. McDermott documents that this period was characterized by intensive economic and community development as well as educational and social reform (8). Such social and economic thrusts are a major factor in abolishing malnutrition, especially if food prices are low relative to the costs of other goods and services. It was also during 1920 to 1930 that pellagra, scurvy, and riboflavin deficiency were almost eliminated as health problems.

George James was acutely aware of the corrosive influence of poverty and malnutrition on health. During his tenure as Commissioner, nutrition services were inserted into prenatal, maternal, child care, and other public health areas. The Bureau of Nutrition operated ten clinics staffed by physicians and nutritionists, which also served in the city's Health Districts. The development of a television nutrition education program for the public on WNYC-TV was another pioneering achievement.

Regrettably, the city of New York's nutrition program and several state Bureaus of Nutrition have decreased considerable portions of their service and research programs. This is an ironic development inasmuch as the recently published Ten-State Nutrition Survey has shown that major portions of our lower socioeconomic populations have documented nutritional deficiencies (9). The impact of further federal fiscal restrictions can only serve to threaten the public health application of substantial nutrition knowledge already on hand. George James knew how to inform the mayor and city fathers of their health responsibilities and by his lucid and logical approach to health problems, also attracted fiscal and professional support for the Department of Health's service and research programs.

### *Nutrition At Mount Sinai*

Nutrition units have traditionally been associated with schools of public health and graduate schools of biologic and agricultural sciences. In the past several years, legislators, lay public and the medical profession have deplored the lack of nutrition teaching in medical schools throughout the nation. While George James was acting Chairman of the Department of Community Medicine, the Nutrition Foundation awarded the Nutrition Division a grant to study the role of a nutrition unit in a medical school-hospital complex. In the ensuing three years, the Division expanded its activities in the community and participated in the following curricular areas: Introduction to Medicine, Biochemistry, Medical Ecology, Physical Diagnosis, Cardiovascular, Infectious Disease and Pharmacology units (10). The elective "Nutrition Problems: USA" has attracted approximately one-third of each freshman class for each of the three years it has been given; recently first-year students have also participated in another elective, "Clinical Nutrition" (11).

Dr. Kurt Deuschle, Chairman of the Department of Community Medicine has expressed the concept that a Department of Community Medicine represents a "third" faculty in a medical school. Analogous to an agricultural extension service which meets the needs of the agrarian community, a Department of Community Medicine can serve the contemporary health needs of urban or rural communities. This philosophy has greatly assisted in the further development of the Nutrition Division.

The Nutrition Division's objectives have followed the George James philosophy of a "problem-oriented" approach to community nutrition needs. Thus, the Division focused on the following basic issues:

1. What are the nutrition-related health problems which can be identified in the communities served by the hospital center and medical school?
2. What priority should be given to these problems?
3. What resources may be identified in terms of personnel, expertise, and equipment which can be mobilized, organized and coordinated in order to successfully attack community nutrition-related health problems?
4. How may we best identify and define the clinical and community nutrition roles of physicians, nutritionists, dietitians, food service managers, social workers, nutrition aides, and others?

As Dean of the Medical School and acting Chairman of the Department of Community Medicine, Dr. James was deeply conscious of the health needs of East Harlem and of the significance of community relationships to the development of the Mount Sinai Center. It was appreciated that nutrition could play a significant role in developing health liaison between Mount Sinai and the community. How was this to be accomplished? How would nutrition be received in the community?

By engaging in "shoe leather" epidemiologic reconnaisance in the community, it became evident that for East Harlem, nutrition was a concept almost more acceptable than health. Health screening was not favorably received in a community which justifiably objected to "one more survey." However, when the nutrition services of the Division were described, defensiveness retreated to cooperation in almost every group approached. It was happily realized that having a nutritionist or dietitian ask an individual about his diet pattern and health characteristics was very different from starkly advising a community group that they needed chest x-rays for tuberculosis screening or that venereal disease control programs were urgently required in the community. We further realized that the provision of nutrition services could be an extremely welcome and effective wedge into the community, spearheading the provision of comprehensive health care. While individuals and communities may argue about the value of family planning, abortions, and the pill versus the loop, few are likely to dispute the positive attributes of "good nutrition." Instead of approaching the community with a threatening, controversial, or negative attitude, namely that they may have a serious disease with judgmental and social implications, the community was provided with the positive attributes of service.

The implementation of the "discovery" regarding nutrition's positive and favorable acceptance by the community was effected through the development of a Community Nutrition Team consisting of a pediatrician and internist, nutritionist or dietitian, and nurse or social worker. The team approached community agencies; jointly they identified high risk populations vulnerable to nutritional problems. This approach was consistent with George James' concepts of health care. As Commissioner of Health, he often encouraged his health officers to develop a "team approach" to local health problems.

The Community Nutrition Team makes home visits, develops storefront nutrition and health clinics (three to date), and gives other consultatory services as the need may dictate. The nutritionist or dietitian obtains a dietary and social history and provides nutrition education where indicated. The physician obtains a medical history, examines the patient, performs venepuncture for laboratory tests and determines whether the patient is to be referred to the Nutrition Clinic, other specialty clinics, or requires hospitalization. The Community Nutrition Team also has the back-up of a Nutrition Laboratory having a spectrum of biochemical indices of nutritional assessment.

### *Nutritional Priorities in East Harlem*

Narcotic addiction is likely the most prevalent and significant public health problem in East Harlem. The Nutrition Division initiated a relationship with the Exodus House Narcotic Rehabilitation Center and established a storefront Nutrition and Health Clinic where 400 former addicts have been evaluated. Their nutritional status has been studied in detail, and coexisting diseases, particularly smoldering hepatitis, have been identified. The participants of this program are rapidly referred to the Mount Sinai Nutrition Clinic, other outpatient services, and are hospitalized when necessary. Many have unusual diet patterns following withdrawal, particularly carbohydrate craving; they also exhibit low folate and $B_{12}$ levels, unusual adipose tissue fatty acid patterns, and almost one-half had abnormal liver enzyme levels and were referred to specialty clinics for further medical evaluation. The community nutritionist, in concert with the hospital's social service, has been the coordinator of field and Medical Center facilities; she has forged an important new role in the coordination of health services for the community. If the resources of Mount Sinai were not made available to Exodus House, comprehensive health care for these young rehabilitated addicts would not have been possible (12). In a joint research effort with the Mount Sinai Methadone Maintenance Treatment Program, we have also completed a nutritional biochemical evaluation of 200 "on the street" addicts prior to entering this program. Once again, serious multiple deficiency states have been identified (13).

Another major priority is school health. The Department initiated health services for about 400 students of the St. Francis deSales Parochial School in East Harlem, who were administered complete physical examinations with special emphasis on nutritional, otologic, psychological, ophthalmologic, dental, and orthopedic evaluations. Seventy percent of this population required and received follow-up treatment. This was a clear indication of the contributions which could be made by the Mount Sinai Center to meet the school health needs of East Harlem.

To George James, community medicine did not primarily involve outpatient care, but a concern for those individuals and families who often sought medical assistance only when pathologic processes were advanced. The Nutrition Division, therefore, sought to ally itself with an agency which dealt with multiproblem families residing deep in the inner city. The Little Sisters of the Assumption were approached. These nun-nurses and social workers, who are expertly trained and dedicated in providing extensive care to inner city families, became the nucleus to which our Division was linked. The Community Nutrition Team established three field clinics with the Little Sisters of the Assumption and, with the assistance of the Social Service Department of Mount Sinai Hospital, the resources of the Mount Sinai Center were also utilized for this population group.

From his personal experience with the Albany Study Group and the Anti-Coronary Club, George James keenly awaited the time when these research projects could be converted into service programs. Albeit on a limited scale, the Nutrition Clinic in the past several years undertook to reverse the nutritional risk factors of 600 obese women. It is often mistakenly assumed that women rarely develop coronary heart disease and that hyperlipidemia and obesity are symptomatic of nutritional affluence. Nutritional evaluation of obese subjects attending the Mount Sinai Nutrition Clinic has indicated that 25 percent exhibit low serum folate, vitamin A, vitamin C, and iron levels, and that many had previously undiagnosed hypertension, hypercholesterolemia or hypertriglyceridemia. The Nutrition Clinic staff has instituted group weight reduction programs utilizing serum cholesterol-lowering diets and a pharmacologic approach to hyperlipidemia when indicated.

On a much larger public health scale, George James was an advocate of a "managerial" approach to lowering high-risk levels of serum cholesterol. Inasmuch as it has been shown that hypercholesterolemia affects up to one-third of men under the age of 45, and that hypertriglyceridemia is also common, the utilization of "food as a vehicle for health" could be attained through the public marketing of meat products enriched with polyunsaturated fatty acids, greater use of skim milk, vegetable oils, cholesterol-free eggs, and the availability of beef cattle raised on feed rich in polyunsaturates. The idea of a "health steak" is currently practical if the food industry and the regulatory agencies of government undertake a redefinition of standard nutritional products (14). This concept is of more than theoretical interest. If developing countries are to evolve nutritional patterns that correct malnutrition but do not result in "national hypercholesterolemia" and obesity by mimicking Western diet patterns, spun soy "meats" and other technologic advances must be implemented.

Along with drug addiction, hypertension among the Black and Puerto Rican population of East Harlem remains a major public health problem. The Department of Community Medicine has recently launched a hypertension screening program which has affirmed the high prevalence of hypertension among the Black citizens of East Harlem. From the work of Dahl and our epidemiologic observations (15), the indictment of excessive salt intake and its reversibility by "managerial" means (desalination of water supplies, low-salt food, nutrition education via biaxial television, etc.), remains another potentially applicable major public health approach to the nutritional control of hypertension.

At the end of the "life cycle" spectrum, the Department of Community Medicine participated in a major health fair at the Covello Senior Center in East Harlem to evaluate 400 senior citizens. Two hundred received a detailed nutritional evaluation which identified special nutritional problems of the elderly. Forty-five percent exhibited hypercholesterolemia, 26 percent elevated glucose levels, 14 percent low hemoglobin levels, and 20 percent were low or deficient in vitamin C. Thirty-five percent had low or deficient folate levels and 20 percent exhibited low serum iron levels. Forty-eight percent of subjects were found to have elevated systolic or diastolic blood pressures. Distance from supermarkets and other food resources, crime in the streets, and lack of accessible transportation, were also recognized as important determinants of the nutritional status of the elderly in East Harlem, many of whom cannot successfully negotiate the difficulties of living in this area.

Exemplifying a problem-oriented, yet integrative public health approach championed by George James, the Nutrition Division has sought to define the paramount nutritional problems of East Harlem. It has even been gratifying that the Division's programs evolved into a "spearhead for comprehensive health care" for these specific population groups. Moreover, despite current federal fiscal constraints which are "throwing away" the failure-to-thrive baby along with the watered budget, the Division is seeking to fulfill the mandate of the community by being a sort of "intensive care unit"—a concept initiated by Dr. Deuschle.

In one man, George James embodied the physician and health administrator who could fully appreciate the contributions of nutrition science and utilize the applied and behavioral sciences to understand how current knowledge could best be applied toward the improvement of the public health. He understood how nutritional risk factors could sharply curtail the epidemic incidence of the major chronic disease public health problems of our time.

Today, medicine stands on the threshold of harvesting the health and social benefits derived from the concepts which George James formulated and applied during his remarkable lifetime of service to his profession and fellowmen.

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# Why Health Economics?

VICTOR R. FUCHS, PH.D.

"The trouble with you economists—" was one of his favorite ways of beginning a conversation. Always skeptical, always challenging, always concerned, he was aware of and interested in any field that might make some contribution to the advancement of health. Economics was no exception. Among physicians in positions of influence and responsibility, he was one of the first to see the relevance of economic research to the problems of health and one of the first to bring economists into medical education on a regular and systematic basis. As first chairman of the Health Economics Advisory Committee of the National Bureau of Economic Research he provided the right mixture of encouragement and caution, insisting always on relevance and rigor. It was most fitting that a recent NBER volume on health economics should be dedicated to the memory of George James (1).

A decade ago health economics was a relatively unknown field. Today it is a flourishing branch of economics with several active centers of research, doctoral training programs at many major universities, and excellent employment prospects in teaching, research, and consultation. Why this tremendous increase in the demand for health economists?

Some of the reasons are peculiar to developments within the health field. They include the rapid rise in medical care prices and expenditures; the development of chronic shortages of some types of services and surpluses of others; the growing concern about equality of access; a heightened awareness of large disparities in health levels within the US and between the US and other countries; and the entry of the federal government into health on a massive scale.

Perhaps more important, though, has been a growing interest in economics generally in our society. Today not only do economists advise about health and medical care, but their help is eagerly sought regarding problems in such diverse fields as housing, transportation, education, and law. What special skills do economists bring to problems in these areas? In most instances, their knowledge of the particular field is limited, compared with that possessed by physicians, architects, engineers, lawyers, and other experts. Their greatest source of strength is in their point of view—their general approach to analyzing any specific problem.

## *The Economic Point of View*

The economic point of view is rooted in three fundamental observations about the world. The first is that resources are scarce relative to human wants.

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It is hardly news that we cannot have everything that we would like to have, but economists emphasize that this basic human condition is not to be attributed to "the system," or to some conspiracy, but to the parsimony of nature in providing mankind with the resources needed to satisfy human wants. That inefficiency and waste exist in the economy cannot be denied. That some resources are under-utilized is clear every time the unemployment figures are announced. That the resources devoted to war could be used to satisfy other wants is self-evident. Still, the fundamental fact remains that even if all these imperfections were eliminated, total output would still fall far short of the amount people would like to have. Resources would still be scarce in the sense that choices would have to be made.

The second observation is that resources have alternative uses. The human, natural, and manmade resources at our disposal can in most instances be used to satisfy many kinds of wants. Finally, economists note that people do, indeed, have different wants, and that there is significant variation in the relative importance that people attach to them. The oft-heard statement "health is the most important goal" is not accurately reflected in human behavior. Everyday in manifold ways people make choices that affect health and it is clear that they frequently place a higher value on satisfying other wants.

Given these three conditions, the basic economic problem is "how to allocate scarce resources so as to best satisfy human wants." This point of view may be contrasted with two others that command considerable support. They are the romantic and the monotechnic.

The romantic refuses to recognize the scarcity of resources relative to wants. The fact that we are constantly being confronted with the need to choose is attributed to capitalism, communism, advertising, the unions, war, unemployment, or any other convenient scapegoat. Because some of the barriers to greater output and want satisfaction are clearly manmade, the romantic is misled into confusing the real world with the Garden of Eden. Because it denies the inevitability of choice, the romantic point of view is ineffective in dealing with the basic economic problems that face every society.

The monotechnic point of view, frequently found among physicians, engineers, and other technologists, is quite different. Its principal limitation is a failure to recognize the multiplicity of human wants and the diversity of individual preferences. Every problem involving the use of scarce resources has its technologic aspects, and the contribution of those skilled in the technology is essential in finding solutions. The solution that is optimal to the physician or engineer, however, may frequently not be optimal for society as a whole because it requires resources that society would rather use for other purposes. The desire of the engineer to build the best bridge or of the physician to practice in the best-equipped hospital is understandable. But to the extent that the monotechnic person fails to recognize the claims of competing wants or the divergence of his priorities from those of other people, his advice is likely to be a poor guide to social policy.

Economists implement their point of view with a set of analytical tools and concepts, such as demand, supply, price, quantity, equilibrium, the margin, and competition (2). The principal purpose of these tools is to enable one to gain a better understanding of the choices people make. An appreciation of the inevitability of choice and a grasp of the variety of levels and kinds of choices is necessary in order to develop intelligent policies for health or any other field. A discussion of some of the most important choices we make, individually and collectively, follows.

### *Health or Other Goals*

The basic level of choice is between health and other goals. While politicians tell us that "health is a right," the realization of that "right" is always less than complete because some of the resources that could be used for health are allocated for other purposes. This is true in all countries regardless of economic system, regardless of the way medical care is organized, and regardless of the level of affluence. It is true in communist Russia and in welfare-state Sweden, as well as in our own capitalist society. No country is as healthy as it could be; no country does as much for the sick as it is technically possible to do.

The constraints imposed by resource limitations are not manifested only in the absence of amenities, delays in receipt of care, or minor inconveniences. They also result in loss of life. The grim fact is that no nation is wealthy enough to avoid all avoidable deaths. The truth of this proposition is seen most clearly in the case of accidental deaths. For instance, a few years ago an airplane crashed in West Virginia with considerable loss of life. Upon investigation it was found that the crash could have been avoided if the airport had been properly equipped with an electronic instrument landing device. It was further found that this deficiency was well known and a recommendation for installation of such equipment had been made several months before the crash. The recommendation was turned down because it was decided that the cost was too high.

Motor accidents take more than 50,000 lives each year in the United States and, because so many of the victims are young or middle-aged adults, the economic loss from this cause is very high. Many of these deaths could be prevented, but some of the most effective techniques, such as the elimination of left turns, are extremely expensive to implement.

Not only are accidental deaths preventable if we are prepared to devote more resources to that end, but so are deaths from many other causes. Within limits, every nation chooses its own death rate by its evaluation of health compared with other goals.

But health is more important than anything else! Is it? Those who take this position are fond of counterposing our unmet health needs with the money that is "wasted" on cosmetics, cigarettes, pet foods, and the like. "Surely," it is argued, "we can afford better health if we can afford hula hoops." But putting the question in this form is misleading. There are other social goals, such as justice, beauty and knowledge, that clearly rank with health and that just as clearly remain unfulfilled partly because of resource limitations.

John Rawls, in his influential book, *A Theory of Justice*, argues that justice is the prime and essential goal for society. His argument is at the theoretical level and concerns far more than courts and law enforcement. Brought down to practical terms, however, we know how far we are from assuring a prompt and fair trial to all accused persons. We also know that part of the answer to getting a fairer and more effective judicial system is to devote more resources to it.

What about beauty, natural or manmade? How often we read that a beautiful stand of trees or some other natural beauty could be saved if a proposed road is rerouted or some other change made (at some increase in cost). How frequently do we learn that a beautiful new building design is rejected in favor of a conventional one because of differences in cost. Knowledge also suffers. Anyone who has ever had to meet a budget for an educational enterprise or a research institute knows how resource limitations constrain the pursuit of knowledge.

What about more mundane creature comforts? We may give lip service to the idea that health comes first, but a casual inspection of our everyday behavior with respect to diet, drink, and exercise belies this claim. Most of us could be healthier than we are, but at some cost, either psychic or monetary. Not only is there competition for resources as conventionally measured, but we are constantly confronted with choices involving the allocation of our time, energy and attention. If we are honest with ourselves there can be little doubt that other goals often take precedence over health. Economists don't tell us which goals to choose but it is their unpleasant task to insist that we can't have everything.

### *Medical Care or Other Health Programs*

Given our individual and collective preferences for health or other goals, a significant range of choices emerges within the health field itself. Assuming we are prepared to devote "X" amount of resources to health, how much should go for medical care and how much for other health programs, such as pollution control, fluoridation of water, accident prevention and the like? There is no simple answer, partly because the question has rarely been explicitly asked. In principle, the solution is to be found by applying the economist's rule of "equality at the margin." This means relating the incremental yield of any particular program to the incremental cost of the program, and allocating resources so that the yield per dollar at the margin is the same in all programs.

Expenditures for any type of health activity, whether for a hyperbaric chamber for a hospital or a rat-control program in the ghetto, presumably have some favorable consequences for health which can be evaluated. It is not easy to measure these consequences, but we could do a lot better than we are doing and thus contribute to more rational decision-making.

An objection that is frequently raised to such a suggestion is that "we can't put a price on a human life". One answer is that we are implicitly putting a price on lives every time we (or our representatives) make a decision about the coverage of a health-insurance policy, the installation of a traffic light, the extension of a food-stamp program, or innumerable other choices. A second answer is that in choosing among health programs it may not be necessary to place a dollar value on human life. It may be sufficient to compare the marginal yield, in lives saved, of different programs to determine that some reallocation of resources would yield significant social benefits.

### *Physicians or Other Medical-Care Providers*

If it were possible to make intelligent choices between medical care and other health programs, we would still be faced with a significant range of decisions concerning the best way to provide medical care—that is, the best way to spend the medical-care dollar. One of the most important of these decisions concerns the respective roles of physicians or other medical-care providers, such as physician assistants, nurse-clinicians, midwives, and family-health workers. A related set of decisions concerns the optimal mix between human inputs (whether physicians or others) and physical capital inputs, such as hospitals, x-ray equipment, computers, and the like.

In short, we should be concerned with the best way to produce medical care and we should be aware that the answer to this question requires both medical and economic expertise. It requires consideration of the relative prices of different kinds of inputs, and of the contribution (again at the margin) of various medical-care inputs to health. The argument that these inputs must be used in some technologically defined proportions is soundly refuted by the evidence from other countries where health systems are successfully using doctors, nurses, hospital facilities and other health inputs in proportions that differ strikingly from those used in the United States.

### *Today or Tomorrow*

One of the most important choices any individual and any society has to make is between the use of resources to satisfy current desires or their allocation for capital-creating activities. Economists call the former consumption and the latter investment.

This broad concept of investment should not be confused with the narrow use of the term in financial transactions, for example, the purchase of stock. Broadly speaking, investment takes place when a tree is planted, when a student goes to school, when you brush your teeth, as well as when you build a house, a factory, or a hospital. Any activity which will confer benefits in the future is a form of investment. To be sure, sometimes a single activity (such as education) will have elements of consumption (that is, provide current satisfaction) along with investment.

Investment can be in physical or human capital. The development of the theory of human capital by Gary Becker, Jacob Mincer, T. W. Schultz and others and its application in fields such as education and health is one of the great advances in the economics of the past quarter century (3). Health is a form of capital—"health is wealth."

Investment in health takes many forms. Immunization, annual checkups, exercise, and many other activities have current costs but will yield health benefits only in the future. Medical education and medical research, both involving expenditures of billions of dollars annually, are prime examples of investment in the health field where the diversion of resources (physicians and other personnel) from meeting current needs is readily apparent.

How much provision for tomorrow at the expense of today should we make? As with all economic decisions, price plays a role either explicitly or implicitly. In this case there is a special kind of price called the rate of interest or rate of return. When decisions are made concerning health investments, we must have some way of taking into account the fact that people discount the future compared with the present. The rate of interest or the rate of return plays that role.

No investment in health will be undertaken unless the investor believes it will yield a satisfactory rate of return. Health professionals frequently despair over the failure of some people to invest in their own health and believe that such behavior is irrational. If a person discounts the future at a high rate, however, as evidenced by a willingness to pay 20 percent per annum interest for consumer loans or installment credit, it would not be rational to make an investment in health that had an implicit return of only 15 percent.

It is abundantly clear that people differ in their attitudes toward the future; that is they have different rates of time discount. The reasons for these differences are not known; they may be related to perceptions about how certain the future is and they may depend upon how strongly rooted is one's sense of the past. Young children, for instance, characteristically live primarily in the present. They lack both a historical perspective and a vision of the future, and their rate of time discount is usually inordinately high.

The importance for health of this question becomes apparent once it is realized that many things that affect health, either for good or bad, have consequences which are realized only after long periods of time. One possible reason why there is such a high correlation between health and years of schooling is that they are both aspects of investment in human capital. The same person who has accumulated a great deal of human capital in the form of schooling may, for the same reasons, have made (or had made for him) substantial investments in health.

Examples of other critical choices that must be made in health, such as the allocation of funds among different medical research programs, are easy to find. It is also easy to say in principle how these choices should be made so as to maximize social utility. The more difficult task is to take the general economic principles and apply them to concrete problems. To do this it is essential to develop a richer, more reliable, more comparable data base for health and medical care.

The development of such a base and its analysis require collaborative efforts by health professionals and economists (4). Such collaboration is becoming easier as economists become more familiar with medical care institutions and technologies, and health professionals broaden their understanding of economics. Despite large differences in vocabulary, temperament, and motivations, physicians and economists do share one important trait—they tend to be realists. Both medicine and economics emphasize seeing people as they really are, as well as what they might become.

An essential ingredient in fruitful collaboration is inspiring administrative leadership. George James provided this in full measure. His realism and his idealism, his ability to get diverse groups to work together, his vision and his dedication, were precious assets. The return on the human capital he helped to create should be worthy of the man and his life.

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# Health Manpower Planning in the Changing Scene of the 1970's

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A central concern of those who plan for the present and future needs of the health care system of the United States is that the supply of health manpower be adequate and well used. There are currently serious imbalances between supply and need, and there is evidence that such imbalances may continue or be even worse.

These problems lead us to ask questions about current capabilities for health manpower planning and for translating planning results into effective policy that would influence the production, distribution, and utilization of health manpower. A further question has to do with the implications of recent policy changes of the federal administration for health manpower.

This paper will discuss these issues with the object of delineating some of the limitations and potentials of health manpower planning and policy-making, and of indicating some of the problem areas that may develop in association with changes in federal health manpower policy.

## *Health Manpower Planning: General Considerations*

In describing the scope of health manpower planning, Baker offers a planning framework that includes: analysis of the current supply of health manpower; projection of the supply forward ten to twenty years, taking into account anticipated additions of new graduates and estimated subtractions for death, retirement, migration, and so forth; assessment of the effective economic demand for health services; projection of demand forward ten to twenty years; evaluation of productivity of health workers in terms such as average number of services provided; and recommendations to effect a balance between projected supply and projected demand, taking into account constraints inherent in the recommendations (1).

While one might want details of this outline clarified (for example, requirements for services might be used to encompass both demand and need), it shows the broad boundaries of health manpower planning.

Such an analytical approach is difficult to apply even under conditions where data on demand are available and planning is closely coupled to administrative implementation. In the United States these conditions are largely lacking, and health manpower planning functions at a considerable distance from this theoretical model when it functions at all.

A fundamental point that needs to be raised about health manpower planning is: To what extent is planning possible in the setting of extreme pluralism that is characteristic of the health system in the United States?

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Reliable data on many aspects of health manpower supply are difficult to obtain, mainly because of the large numbers of educational and service institutions that function independently of one another and of regional or national bodies that might keep track of the supply, and because there has not been a well-financed and coordinated attack on the problem. Determining effective economic demand for health services or for the manpower required to provide those services in a changing health care system is exceedingly difficult under conditions in which health services are not the product of a national health plan with relative uniformity across the nation, as in many countries, but a summation of a variety of approaches at local, state, and federal levels. Developing policy and implementing mechanisms that would attempt to establish a balance between supply and demand are difficult not only because data on supply and demand are difficult to obtain but also because neither educational programs that add to the supply nor services that respond to the demand are the direct product of national decisions but, again, are the result of local and state as well as federal initiatives.

The interrelationships among the various levels, federal and nonfederal, at which health manpower planning and policy-making might be carried out are also important. On the one hand, the multiplicity of training programs, mobility of health personnel, and variations in regional need require a national overview of supply and requirements. On the other hand, the variations in regional and local supply and requirements call for planning and policy-making at those levels. A current problem is the limited planning capability at all levels, particularly regional and local. The need for improving on those capabilities is clear, as is the need for building toward a network of health manpower planning activities in which, ideally, methodologies and information would be shared and policy-making and implementation would be balanced among the levels.

Realistically, the components needed for planning and policy-making in the health manpower arena will not come together in a neat set of relationships. Data on supply and methods for determining requirements need to be substantially strengthened; even so there will remain areas of great uncertainty. From a policy point of view, the need is to couple indications of trends derived from the best data available with the interests of legislators, educators, employers, and special interest groups, in order to put together a set of forces adequate to change policy and programs either on the supply or the demand side of the health manpower equation.

Recent announcements of the national administration point to dramatic changes in the federal role in health. These changes, if implemented, include substantial federal withdrawal from direct funding of health manpower education programs in favor of guaranteed loans to students who would seek educational programs according to their own choices. Similarly, there may be a trend away from federal support of specific health care models, leaving it to the population, supported to a greater or lesser extent through health insurance, to seek health care according to personal choice. The overall trend would seem to be toward more of a free market system of the production and utilization of health manpower. These changes are clearly of major importance to any consideration of the health manpower sector.

This discussion will now proceed to a more detailed consideration of problems associated with monitoring the health manpower supply, determining manpower requirements, modulating or controlling the production and distribution of health manpower, and of some policy implications of these problems.

### *Monitoring the Health Manpower Supply*

Our national capability for monitoring the supply of health manpower varies greatly depending on the level and type of health personnel.

At the professional level, data originate from a variety of sources including professional organizations, census reports, immigration data, and educational institutions. None of the data are dependable in themselves; it is necessary to use multiple sources in developing approximations. For example, working out the total numbers of foreign medical graduates in all settings involves estimates based on several sources of data. Legislation providing capitation payments for professional undergraduate educational programs has included mechanisms for acquiring precise data on current and projected manpower production that have added considerably to the quality of professional health manpower data. It should be noticed, however, that the data are provided largely because of the financial leverage inherent in federal support of the programs, and that the figures are for the output of undifferentiated health professionals. This particular federal control mechanism, therefore, does not touch the problems of specialization and distribution, which are at least as important as absolute output.

Measurements of nursing manpower are less dependable than those for the health professions mentioned above. Among other reasons, it is harder to get reliable data on one million nurses than, for example, 5000 podiatrists. Most of the information comes from the Division of Nursing of the Bureau of Health Manpower Education in the National institutes of Health and from the National League of Nursing. The federal government has been providing modest capitation payments for nursing, but only about two-thirds of 1400 institutions involved in nursing training have opted for them. Thus, the data available in the nursing area seem to be of modest quality (2).

Obtaining information on the fields of allied health manpower is difficult because of the recent multiplication of subspecialities, job titles, and formal training opportunities and the lack of consensus for defining categories and functions of allied health workers. Contributing to the problems of collecting data is the large number of institutions involved—more than 2,000, compared with 1,400 schools for nursing personnel and 270 for health professions (3). Many of these institutions for training allied health workers are not affiliated with university training centers and some do not have hospital connections. Rather than there being a single national professional organization with which to deal, there are a number of organizations that cover only part of the field. Relatively little money is flowing from federal sources directly into these programs and little information is flowing directly back to federal sources. Thus, historical information is scarce and the collection of uniform data is difficult, yet allied health workers represent one of the nation's most important health resources.

Although our current national capability for monitoring health manpower is limited, substantial efforts are underway at a number of levels to develop the necessary know-how and institutional structures. The Division of Manpower Intelligence (DMI) has recently been established in the Bureau of Health Manpower Education (BHME) of the National Institutes of Health in the Department of Health, Education and Welfare (DHEW). Its purpose, stated most simply, is to develop the information systems and technologic capability needed for surveillance and analysis of the state of the nation's health manpower resources and requirements, both current and future. An example of the capabilities of the DMI is its publication, "The Supply of Health Manpower: 1970 Profiles and Projections to 1990," which is currently being reviewed and revised and will probably be available later this year (4).

The DMI relates to the Secretary of DHEW through the offices of the Assistant Secretary for Health and the Assistant Secretary for Planning and Evaluation. While many other federal offices and bureaus have responsibilities for health manpower—144 programs for health manpower operate out of 13 separate federal departments and independent agencies—these three offices would appear to have the primary or focal responsibilities for assembling the information and concepts needed for developing a national health manpower strategy. On the other hand, because of the most recent announcements of the administration that indicate cutbacks in federal support of health manpower education programs, it is unclear what level of support this focus of health manpower planning within the federal governmental structure will have.

Efforts at nonfederal levels to monitor health manpower supply are underway —at municipal, state, and regional agencies, and national professional organizations—but the data produced are uneven in coverage and quality. Indeed, a major obstacle to decentralizing health manpower planning is the lack of local organizations to which to decentralize and the lack of local and state data on which to base planning activities.

### *Determining Health Manpower Requirements*

Determining manpower requirements for an evolving health care system is exceedingly difficult. Not only are the data inadequate, but there is also the extreme complexity of the underlying analysis of supply, need, and demand. Estimates of future requirements cannot be based solely on analyses of current staffing patterns and related shortages, yet it is difficult to project the future patterns that will evolve. These difficulties are illustrated by the effort of the Division of Allied Health Manpower of the BHME. In projecting manpower requirements at a national level, they found it necessary to restrict the meaning of 'requirements' to the number of workers who could find employment over the coming decade without major changes in the health system, manpower utilization patterns, or in the economics of health care. Further, these estimates were largely built on expected increases in the gross national product and the estimated percentage devoted to health care (5). Of course, as they acknowledge, these parameters are constantly changing, and to estimate the number and variety of workers necessary to achieve some improved level of national health, taking into account the dynamic nature of the health care system, manpower utilizations patterns, the economics of health care would involve and probably exceed our utmost technologic capability.

A number of methods have been used for determining or projecting health manpower requirements, including: ratios of health manpower to population; ratios of health personnel to enrolled populations in prepaid group practices; estimates based on professional judgments of health manpower required to provide a defined level of care for a specific age group or for specific illnesses; functional analysis through which numbers and qualifications of personnel are systematically matched to health care requirements; and so forth (6–17).

The Division of Manpower Intelligence has been developing several health manpower requirements and supply models. One model includes seven basic components: (1) the population to be served, including needs and demands for care; (2) the organizational structure of the health care delivery system; (3) the utilization of health care services in each organizational structure; (4) manpower mixes required to serve these organizations; (5) educational production and additions from immigration of health manpower supply; (6) locational and specialty distribution of health manpower; and (7) implications of future developments for each of the preceding items (18).

Each of these approaches to the determination of health manpower requirements has limitations, and none can serve alone as a dependable basis for manpower policy determination. A number of reviews describe the complexity of the subject, identify shortcomings in the methodologic approaches, and point out new directions that might be taken (8, 18–21).

### *Modulating or Controlling Health Manpower Production and Distribution*

Having noted the limited quality of information on health manpower supply and the early state of development of the art of determining health manpower requirements, it should also be appreciated that mechanisms for translating these data into effective policy are generally lacking. An important question, therefore, is: To what extent is it possible or desirable to modulate or control health manpower production and distribution and what mechanisms are available for doing so?

We will begin by asking to what extent the health system is self-regulating with respect to the supply and demand of health manpower. This question is particularly appropriate in view of the apparent trend of the national administration to withdraw federal support from health manpower educational programs in favor of greater dependence on a free market system. We will then consider other approaches, both federal and nonfederal, to influencing health manpower production and distribution.

The market mechanism, in theory, works automatically with no need for government to intervene or plan. Operating through individual purchase and sale, with prices determined by competitive forces, it enjoys the advantages of reliance on individual responsibility and self-interest, impersonal determinations, and freedom from bureaucratic control (22).

In the manpower field, however, the market mechanism has significant shortcomings: the market may fail to supply information and guidance necessary for rational career and job choices; employment, training, transfer, and promotion are often highly administered matters not easily subject to market dynamics; the market mechanism may stress immediate gains at the expense of serious long-term losses; the market may provide local information that is at variance with national and other local area needs; and the market has proved deficient as a means of preventing racial and sex discrimination in employment (22).

Such shortcomings of the market mechanism apply to health manpower as well and appear to vary with the level of manpower. At the professional level, supply often tends to create its own demand. Surgery is the popular example—surgeons are able to find surgery to do, even where there is a surfeit of surgeons (23). With respect to shortages of professional manpower, the time lag between a signal that there is a shortage and a responsive increase in production from educational programs can amount to several years. Local or regional shortages, particularly in areas at lower socioeconomic levels, may not represent a force adequate to attract a supply of professionals. Finally, there is the familiar issue that demand does not necessarily correspond to need; that is, the health problems of a population may call for forms of care that are different from those that they are asking for.

At the level of allied health personnel, a free market mechanism may be better suited to maintaining a favorable relationship between supply and demand. These persons may be less able than professionals to create demand through their availability. Maldistribution may be less of a problem since they often concentrate where the need is the greatest; namely, the areas of the relatively poor and underserved, but not usually to the extent that alleviates their maldistribution. The time lag between sensing over- or undersupply and responses of training programs can be expected to be much less than with professional personnel (2, 24).

Nursing personnel generally follow a pattern of distribution similar to that of physicians, but as secondary family workers they tend to be less mobile geographically than physicians, and to live near the sites of their training. It seems to be easier for an area to generate a stable population of nurses than of physicians (24–26).

Apart from a free market mechanism, which can be expected to function with limited effectiveness, what other ways might be employed to improve imbalances between supply and requirements for health manpower?

At the federal level, the major level for influencing the supply of health manpower is funding. Increased production of health personnel follows from increased funding. As noted previously, this funding leverage has been greatest with professional personnel, modest with nursing, and negligible with allied health personnel.

This form of federal control of health manpower has been directed toward simple quantitative output. To influence the geographic and specialty distribution of health personnel is another and considerably more complex problem, and while bringing any influence to bear on these parameters is difficult, opportunities exist for both federal and nonfederal action.

It is useful at this point to use physicians to illustrate maldistribution with respect to location and specialty and the difficulties and opportunities involved in influencing that distribution.

### *Physicians As a Case Study in Health Manpower Production and Distribution*

For physicians there are few barriers to choice of either location or specialty, and there is an unevenness of distribution with respect to both. We will first document the extent of that maldistribution and then proceed to consider the ways in which the patterns of production and distribution might be influenced.

That there is geographical maldistribution is clear. Physician/population ratios in 1970 ranged from 196 and 190 per 100,000 population in the Middle Atlantic and New England states, respectively, to 132 and 105 per 100,000 in the West South Central and East South Central states, respectively. Individual state ratios ranged from a high of 236 per 100,000 in New York to a low of 89 per 100,000 in Mississippi (27).

Ratios of health personnel to population are, in many respects, an inadequate index by which to describe the distribution of health personnel (19), but they do allow rough comparisons. Ratios derived from large areas such as states and cities, however, can obscure more localized distributional differences. In Baltimore, for example, which has a favorable overall physician/population ratio, among 15 study districts the numbers of practicing physicians per 100,000 population ranged from 25.8 to 504.1, and 15 census tracts in the inner city totally lacked primary care physicians (28). Similar gaps apply to rural areas as well. As of 1970, there were 132 counties in the United States with no active nonfederal physician. Those counties represent four percent of the land area of the nation and are populated by nearly one-half million people (29).

Maldistribution with respect to specialty is also evident, though less immediately obvious than that of geography. To begin with, there is the well-known trend toward specialization as such. In 1931, only 10 percent of all physicians were engaged in specialty practice; by 1970, the figure had increased to over 80 percent. While the numbers of physicians in specialties has increased steadily in recent years, the distribution among specialties has changed relatively little, the most significant changes being in internal medicine and pediatrics. The proportion of all physicians in internal medicine increased from 11.6 percent in 1963 to 13.5 percent in 1970. For pediatrics, the proportion changed from 4.7 to 6.1 percent. During this same period there was a decline in general practice from 26 percent to 13.5 percent of all physicians, a decline that is partly offset by the increased proportions of physicians in internal medicine, pediatrics, and obstetrics and gynecology, who devote a substantial part of their time to primary care (30).

Medical specialists are also maldistributed geographically. Comparisons of California and Alaska will illustrate. In California there is one internist for every 3,500 people, whereas in Alaska there is one per 14,400 people. There is one surgeon per 7,100 people in California and one per 12,500 in Alaska, and one pediatrician per 10,000 in California and one to 20,000 in Alaska (31).

Given these figures for specialty distribution, how does one judge their appropriateness? As noted earlier, our capability for determining health manpower requirements is very limited, and to do so for physicians is no exception. For the purposes of the current discussion, two methods for estimating requirements for physicians will be considered briefly, one based on professional judgments of need, the other using existing patterns of physician-staffing in several prepaid group plans. These two methods are not being presented with the intent of analyzing them or of recommending one over the other, but to assist in identifying some important issues in health manpower planning and policy.

Schonfeld et al estimated the number of physicians needed to provide primary medical care of "good quality" to be about 133 physicians per 100,000 persons of all ages in the population (excluding dental, mental, and obstetrical care, and routine physician examinations for adults). These estimates were based on the professional judgments of internists and pediatricians engaged in providing primary care and on the prevalent patterns of performance among community physicians engaged in solo or partnership practice. They estimate that about 65 nonfederal physicians were available for primary care in 1966 per 100,000 persons, and about 59 per 100,000 in 1970. They discuss ways of narrowing the substantial gap between need and supply of physicians and point out that this manpower gap is not between demand and supply but between true clinical need and supply (11).

A second method uses the ratios of physicians to enrolled populations in existing prepaid group plans on the assumption that these plans have arrived at satisfactory physician staffing patterns on a pragmatic basis. Mason presents "optimum physician-population ratios for 18 specialties based on experience of six prepaid group plans." The physician/population ratio for all six groups was 1:1061, which can be compared with a national physician/population ratio of 1:766 (32). Looking at the physician/population ratios by specialty, there is a considerable range in the ratios among the plans due to local staffing patterns, but, nonetheless, the data are useful for identifying current patterns of physician staffing. Tables I and II show the average ratios of specialist physicians to population for selected specialties in the six plans.

Since Schonfeld et al estimated a need for about 133 physicians per 100,000 of population to provide primary care (11), it is instructive to look at comparable figures in these prepaid group plans. Family practitioners, internists and pediatricians together (omitting obstetricians and gynecologists in order to

TABLE I

*Ratios of Specialist Physicians to Population Enrolled in Prepaid Group Plans, Averages for Six Groups**

| Specialty category | Physicians/100,000 enrolled population† |
|---|---|
| Family practice | 23.8 |
| Internal medicine | 27.3 |
| Pediatrics | 17.1 |
| Obstetrics and gynecology | 10.3 |
| General surgery | 8.1 |

* The group plans are: Health Insurance Plan of Greater New York; Kaiser-Permanente Plans of Portland, Oakland, and Los Angeles; Puget Sound; and Group Health Association, Washington, D.C.

† In obtaining the above data, the original physician/population ratios from Mason (31) were converted to physicians per 100,000 of enrolled population and weighted according to the populations enrolled in each plan; for each specialty a weighted average of physicians per 100,000 population for all six plans was then calculated. If a particular type of specialist was not represented in the plan, it was excluded from the computations.

TABLE II

*Ratios of Surgical Specialists to Population Enrolled in Prepaid Plans, Average for Six Groups**

| Surgical specialty | Physicians/100,000 enrolled population |
|---|---|
| General surgery | 8.1 |
| Obstetrics and gynecology | 10.3 |
| Neurosurgery | 0.9 |
| Ophthalmology | 2.7 |
| Orthopedics | 3.8 |
| Otolaryngology | 2.2 |
| Plastic surgery | 0.2 |
| Urology | 1.6 |
| Total, surgical specialties | 29.8 |

* Explanatory notes as in Table I.

maintain comparability with the Schonfeld data) amount to 68.2 physicians per 100,000 enrolled population, roughly half the need estimated by Schonfeld et al. The number derived from the plans is probably high, since not all the work of internists and pediatricians involves primary care. Thus, the estimates of Schonfeld et al are at least two-fold those actually used in the operation of these prepaid group plans.

There are distinct limitations to each of these approaches to determining requirements for physicians. For the present, we can simply note these requirements defined by each approach and that there are substantial differences between them.

TABLE III

*Physician/Population Ratios in Medical Specialties: in Prepaid Group Plans and Nationwide, actual and projected, 1980 and 1990*

| Specialty | Physicians/100,000 population enrolled in prepaid group plans, 1970* | All active physicians per 100,000 population, United States† | | |
|---|---|---|---|---|
| | | 1970 | 1980 | 1990 |
| General practice | 23.8 | 28.4 | 19.3 | 12.5 |
| Internal medicine | 27.3 | 20.5 | 30.1 | 40.6 |
| Pediatrics | 17.1 | 9.2 | 12.6 | 16.3 |
| Obstetrics and gynecology | 10.3 | 9.3 | 10.8 | 12.8 |
| General surgery | 8.1 | 14.6 | 23.5 | 32.8 |
| Surgical specialties‡ | 29.8 | 41.9 | 56.2 | 73.0 |

* See Table I for explanatory notes.

† Taken from The Supply of Health Manpower, 1970 Profiles and Projections to 1990. (Interim Report) No. 73-44 BHME/DMI/MRR. Table 37, p. 136. U.S. Department of Health, Education and Welfare, National Institutes of Health, Bureau of Health Manpower Education, Division of Manpower Intelligence. October 1972.

‡ See Table 2 for list of surgical specialties.

Table III shows actual physician supply in the United States by specialty with projections of the supply to 1980 and 1990, developed by DMI, together with the data from the prepaid group plans already discussed. The technical difficulties of such forecasting are well known. The assumptions and methodologies of the projections are described in the source materials and will not be repeated here except to say that the major policy assumption was that funding, federal or otherwise, would be available through the projection period at a level adequate to support the productive capacity and enrollments expected to result from the Comprehensive Health Training Act of 1971 (33). First, comparing staffing patterns of the prepaid group plans with actual supply, there are areas of undersupply, particularly in pediatrics, and of oversupply, as in surgery. The actual supply of what might be called primary care practitioners (general practice, internal medicine, pediatrics, perhaps obstetrics and gynecology) is close to the requirements of the prepaid group plans.

Looking at the projections, some of the undersupply will be corrected by 1990, as in pediatrics, but the level of primary care practitioners will change little over the coming two decades, the increase in internists and pediatricians being balanced by a decrease in general practitioners. However, recent information indicates the possibility of a substantial increase in the very small field of family medicine (2). The oversupply of physicians doing surgery will not be corrected but may become exaggerated.

Clearly, these data and the assumptions and methodologies on which they have been based require critical study, and caution should be exercised in applying results. Nonetheless, the following points can be made:

Two approaches to determining requirements for physicians differ substantially—a full 100 percent. Those differences extrapolated to a nation have important implications for manpower planning and policy. Next, whatever method is used for defining requirements, it is likely that there will be areas of marked oversupply and undersupply. Again, extended to the nation, those degrees of mismatch between need and supply are extreme.

Having examined data on the supply and distribution of physicians with respect to both location and specialty, and having noted examples of serious maldistribution, we can ask to what extent these factors are subject to influence or control.

Physician choice of location in which to live and work seems to be influenced mainly by: presence or absence of hospitals and training facilities; degree of urbanization; per capita income levels; and where the physician was born and educated, particularly where he had his residency training (34). Joroff and Navarro studied the relationship between ten community characteristics and the distribution of physicians in urban United States and found that the best predictor of general practitioner rates was the percentage of the population aged 65 years and over, that medical specialties were most strongly related to the existence of a medical school, and that the general hospital bed rate was the best predictor of surgical specialty distribution (35).

The choice of a particular specialty appears to follow from the personality and background of the student, the influence of the medical faculty, the availability of residency openings, and the provision of federal monies as an added incentive to enter particular residences (36).

Thus, while factors influencing choices of location and specialty are complex, the residency appears to be an important variable. It is useful to examine the interrelationships between residencies and the issues relating to influencing or controlling health manpower distribution.

One impressive fact is the widespread availability of residencies with virtually no barriers to entry. Historically, residency programs have developed around the needs of hospitals to provide care, particularly for inpatients. Other factors include the need to instruct medical students, interests of clinical departments in maintaining or expanding their programs, and a general unwillingness to use specialty accreditation as a means of influencing the specialty manpower supply (37). The overall health care needs of the public, generally speaking, have not influenced the pattern of residency programs.

Examining the costs of these training programs and the way in which they are paid for reveals possibilities for influencing the production and distribution of health manpower. The aggregate annual training costs for interns and residents in this country were estimated to be about $670 million in 1970 and increasing. (The comparable figure for nursing is about $500 million (38). These figures for graduate education can be compared with the cost of undergraduate medical education of roughly $400 million per year.) What part of these costs represents training and what part service is not clear, but the overall costs are subsumed under the costs of patient care and form a highly visible $8 per day (3).

Third-party payers and the public have been restive over the inclusion of these training functions in the costs of patient care. The Commissioner of Insurance of the State of Pennsylvania, for example, has tried to exclude such costs from the rate structure of Blue Cross.

If these costs were to be removed from the cost of care and funded separately using federal or state sources, an avenue for influencing or even controlling the numbers and types of internships and residencies and, to some extent their distribution, would be created. A similar concept could be applied to the production of nursing and allied health manpower. The leverage for these fields might come through funding of the clinical practicum that is an essential part of these programs.

Controls of this type would be met by staff opposition, particularly from hospitals dependent on these personnel for essential services. There are conflicting needs between hospitals for training programs that provide personnel for their own internal functions, and the health manpower requirements of a more broadly based health care system that attempts to meet needs of populations. These conflicts could lead either to hospitals evolving in the direction of greater involvement in new forms of health care (as integral parts of a health care network, for example), or to the development of new institutions to produce the health personnel society needs.

While primary responsibility for intern and residency training programs has resided with hospitals, universities have played an essential role in the development of and have contributed to the quality of those programs. It may be expected that universities, with their affiliated hospitals, may take the lead in these new directions of health manpower training.

Looking to the future, assuming a national health insurance program, and given a decision to provide direct support for these training programs, it might be possible to use trust funds associated with the insurance program for support of the facilities and training programs for health manpower necessary to operate the health care system.

Whether the federal government could handle the complexities of assessment of health manpower production and requirements in order to implement such a mechanism is an open question. Whether or not it should is another question.

Turning to other mechanisms for influencing production and distribution of health manpower, to what extent might voluntary action by educational institutions, hospitals and professional groups be counted upon to take action? For example, if a health manpower information system could signal an impending overproduction of certain categories of health personnel, would the educational institutions voluntarily cut back their programs, or would they wait for the fact of oversupply to be felt? Historically, the Cassandras have had limited effect. Alan Carter, the labor economist, predicted in 1965 that the production of Ph.D.'s would reach equilibrium with need in 1970. It is difficult to know who was listening, but few responded. We now witness government cutbacks in stipends for Ph.D. programs and an enlarging number of unemployed Ph.D.'s.

Voluntary action might be taken by the major medical associations such as the American Medical Association, the American Association of Medical Colleges, and various specialty boards, whose policy makers are concerned with modulating the production and distribution of health manpower. An example of professional associations taking leadership in this area is provided by surgery. Francis Moore, in his presidential address to the American Surgical Association, has urged the surgical profession to "take the lead by providing new controls and strong guidelines for the practice of medicine and surgery" in the United States before the federal government does. His proposals include validation of the credentials of surgeons on hospital staffs, monitoring and controlling the flow of surgical residents by a nationally respected nongovernmental body, and establishing a national surgical placement service to identify locations in need of surgeons and surgeons in need of locations (39).

These observations on the supply and distribution of physicians can be summarized by stating first, that there appear to be serious imbalances between the supply and requirements for physicians, both current and projected, and, second, that while there are mechanisms for influencing the output of physicians, there are essentially no effective means of influencing their distribution by location and specialty.

## Conclusions

This is a critical time in the evolution of health manpower planning and policy in the United States. Several forces have converged to form a pattern that has important implications for health manpower and its role in our evolving health care system.

First, there is the slowly growing capability to grapple effectively with the technical aspects of health manpower planning. The capability for monitoring the production and supply of health manpower with reasonable effectiveness is fairly close at hand, as witnessed by the work of the Division of Manpower Intelligence of the National Institutes of Health and research workers in the field. Forecasting what the supply will be in future decades is complex and requires a number of assumptions about which questions can always be raised, but, nonetheless, useful alternative projections can be developed. It is in determining health manpower requirements for our evolving health care system that our technical capability is relatively undeveloped. Taken together, these deficiencies in health manpower planning technology render us vulnerable to the development of serious mismatches between supply and demand or need. Indeed, there is evidence at hand to suggest that such mismatches are with us now and will become more serious in the future, oversupply of surgeons being the most obvious example. The costs of such mismatches, in dollar terms of large investments in the production of nonessential health personnel, and in human terms of inadequate services when there is serious undersupply of health personnel, are potentially great.

It needs to be appreciated, of course, that data and planning technology alone, however refined, may not result in effective policy, or, for that matter, in any policy at all. Even crude data showing an important trend can coalesce the interests of, say, legislators, professional societies, employers and educators, leading to new policy and programs. Nonetheless, improved approaches to health manpower planning increases the likelihood that policy makers will have a dependable basis for their decisions, particularly when undesirable trends are anticipated with enough lead time to allow effective remedial action.

Second, recent decisions by the federal administration to draw back from funding in wide areas of health manpower training could have at least two effects that are important to the present discussion. One is the likely cutting back of federal funding, within the government and extramurally, of both research on health manpower planning methodologies, and the development of operational health manpower planning and policy programs. The other is a possible decreased interest at the Federal level in monitoring and modulating health manpower production and distribution, leaving the free market mechanism to be a major factor governing the dynamics of health manpower production, distribution, and utilization. Against the background of these developments, it is important to note, as described above, that free market mechanisms can be expected to work with limited effectiveness in the health manpower sector.

Given these trends, it is possible that our national capability for effectively influencing the production and distribution of health manpower to match the health care needs of the population could not only cease to progress, but could actually regress. It is critically important, therefore, that there be continued investment, from non-federal sources if need be, in the further development of health manpower planning technology, and in ways of translating planning results into effective policy, including the exploration of mechanisms, nonfederal as well as federal, for monitoring and influencing or controlling production and distribution of health manpower. At the least, there should be an alert monitoring of the overall process of supply and requirements in order to sense the development of further mismatches between health manpower supply and needs of the American people.

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# Government and Health

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To most health care practitioners, government is like the elephant to the blind men; depending on where each one happened to touch, he decided it was a rope, or a wall, or a pillar. In his extraordinarily varied career, George James felt the impact of government on health in all, or substantially all, its aspects. It seems an appropriate tribute to him to attempt to catalogue and in some fashion to order the multifarious impacts of government on health, so that its elephantine potential, and its elephantine limitations, can be more clearly recognized.

A physical model of governmental roles in health would have to be at least three-dimensional. Along one axis one would find the main branches of health care: education and training, research, and delivery, each with its various subdivisions. Along a second axis one would find the variety of governmental functions: as direct consumer and producer, as regulator, as payer, and as exemplar. And along the third axis one would find the various levels of government: local, state, national, and international. Individual three-dimensional models might be constructed for various populations: the poor, the middle-class medically indigent, the old, the young, the city-dweller, and the rural resident; for various kinds of health care, medicine, dentistry, public health, mental health, and for the specialties and subspecialties within each broad field. Lastly, one would have to find a place in the picture for the several roles of government as they directly affect the level of health in the country and in the world, whether by admitting foreign medical graduates who may be more needed at home, labeling cigarette packages, imposing safety requirements on automobile manufacturers, dealing with the social consequences of alcoholism and drug abuse, or sending disease-resistant seed, or DDT, or IUD's, or iron bombs to Asia.

What I propose to do here is first to try to put some flesh on this very skeletonic elephant, and then to see if I can develop some tentative conclusions about its habits and potential demeanor vis-à-vis the health care professions.

To begin with governmental roles, the government is first of all a consumer of health care services for limited populations of its employees, a consumer of limited quantities of training for health care professionals in its employ, and a consumer of certain research designed to answer questions of specific interest to government as it goes about its daily business. The line is hard to draw between the government's role as consumer, and as general sponsor, particularly of research and training. Similarly, there are distinctions between services provided in kind, such as to the military, or offered to be paid for by government under a variety of options, such as to federal civil servants. But there is no particular reason to draw a sharp line here. As a consumer, the government is not significantly different from other larger employers or large private enterprises, but it has at least as much opportunity and more incentive than private employers to experiment with new ways of purchasing health care.

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The government is also a producer of health care, in the military services, in the Veterans Administration, in the hospitals and laboratories of the National Institutes of Health, in state mental hospitals, and in penal institutions at all levels of government. The quality of this care can vary enormously; but again, it is produced on a relatively limited scale. The scale is large enough, as in the case of the Veterans Administration, to provide interesting opportunities for experimentation and modeling (discussed further under the role of the government as exemplar), but still not large enough to restrict the opportunities in the private sector—unlike the national health services of other countries.

As regulator, government plays a more traditional, and much more pervasive role, and at every level from local to international. The licensing of various health care professionals, and the formulation of distinctions among their responsibilities are major regulatory functions of state government. The federal government is concerned with licensing medicines, while state and local government are concerned with licensing or regulating health care institutions (hospitals, nursing homes, health professions schools). Some regulations are quite detailed (prescribing how far below the gum line the dental assistant may probe with her (his?) cleaning tool); some are quite general and even loose (prescribing the standards for operation of nursing homes for the elderly). Regulation also varies considerably in the degree to which government serves primarily as conduit for professional interests, or as protector of the general public interest. There are a number of areas in which accreditation of institutions or licensing of individual practitioners remains in the hands of private professional groups, with little or no government supervision.

Increasingly, the regulatory power is a concomitant and consequence of the government's role as payer, funding, in whole or in part, the operations of particular kinds of health care delivery, training or research institutions in the private sector, although much less so at the state and local level than at the federal level. The standards established by the Medicare and Medicaid programs serve to a significant degree as regulatory measures for the institutions receiving federal funds.

The role of government, primarily the federal government, as a major source of funding for health care in all its aspects is a comparatively recent phenomenon. Less than 20 years ago (in fiscal 1955), public spending on health care (including education and research) amounted to $4.4 billion, or 4.6 percent of total spending on health care, while the comparable figure for fiscal 1971 is $28.5 billion, or 38 percent of total spending. Most of the increase resulted from the direct subvention of health care through Medicare, but federal funds have successively supported expansion of numbers of hospital beds, basic and clinical research, education in the health professions, and modest attempts at new kinds of ambulatory care delivery institutions. The rationales of federal funding programs have differed widely, from what is essentially income redistribution, or helping disadvantaged segments of the population meet their health care bills, to support for long-range social infrastructure that could not find adequate support in the market place or from private contributions.

Despite the tendency of some federal budget officers to speak of "the health dollar" as if it were made of interchangeable parts, there is little evidence that even at a single level of government the various funding programs in health care are related in a consistent overall strategy or program. Specifically there has been no clear effort to relate spending for delivery of care to spending for education and for research—and indeed the Assistant Secretary for Health has no authority over the biggest pieces of health care delivery spending, in Medicare and Medicaid. The relative roles of organized health care professionals, consumers, and government officials also differ considerably from program to program, and there is no recognized standard by which to judge their levels of participation in decision-making. Majority consumer participation is required, for example, under Comprehensive Health Planning legislation, but not under the Regional Medical Program. At the same time, government funding originally intended to support one aspect of health care, i.e., research, has become an essential element in the support of another aspect—education. Not only have certain sectors of health care become dependent on government funding not originally intended for their support, but the irregular pattern of government funding has produced distortions in health care activity, directly responsive to that pattern, from imbalances between hospital and ambulatory facilities to overconcentration on specific kinds of research, and overproduction of certain kinds of specialists.

Government as payer shades off in one direction into government as regulator, and in another direction the payer function shades off into the exemplar function. Government can use the threat of withdrawal of payments as a stick, or it can use the offer of payment as a carrot to create an exemplary program. The federal government in particular undertakes or funds programs intended as demonstrations or examples, which, if they succeed, may be launched on a larger scale with public, private, or, more likely, mixed public and private funding. Neighborhood health centers and family practice residencies are enterprises that may be chosen for demonstrations before any general commitment to public support is made. Difficulties may arise because a successful demonstration is still no guarantee of larger-scale support, particularly if popular or governmental interest shifts away from the population most likely to be benefited by a wider application of the demonstration program.

In addition to these various forms of support for health care programs, government addresses national and local health levels more directly by regulating conditions that affect mortality and morbidity in industrial practices, environmental conditions, and in marketing of goods and services. Some of this regulation is direct, and some of it is quite indirect.

Any government actions that affect the health of the economy also affect the health of the nation, and while an affluent society has its own diseases, from obesity to hypertension, they seem to impact particularly on the less fortunate members of that society. We tend to think of the less acute forms of mental illness, for example, as the prerogative of the well-to-do, but this may be simply because we accept manifestations of emotional pathology in lower-class society as normal behavior under the stresses of their socioeconomic situation, or depending on the political outlook of the observer, of the true inwardness of their nature. At some levels of the Gross National Product, government actions that reduce inequalities of income are probably more important for health than government actions that increase GNP. And just as the general level of health in a society is more likely to be affected by changes in the general conditions of that society than by changes in the level of health care, so government is likely to have a greater affect on levels of health through its general activities than through its health care activities.

There is an interesting paradox here. As government comes to play a larger role in the health of the nation, quite outside the health care system, that system has grown to the point where it is one of the primary concerns of government. The reasons why health care has become the largest industry in the United States need not be unraveled here. Clearly they include the development of science and technology, the increasing affluence of the society, a greater feeling for the value of human life (perhaps as a result of the first two developments) and perhaps also the perpetuation of organizational arrangements that made the health care industry one of the less efficient major industries in the United States. But in any event, health care has become a major governmental concern if only because it bids fair to be the major economic activity in the country.

Another way of looking at the role of government vis-à-vis health care, therefore, is to compare it to the role of government vis-à-vis other major and even minor industries. We can characterize the principal roles of government in its relations with industry as a source of subsidies, a provider of technical assistance, a guardian against monopoly power, and a regulator of business practices.

By and large, the practitioners in the health care professions do not recieve the kinds of direct subsidies that go, say, to the oil industry, or to the housing industry (at least to that portion that builds low- and moderate-income housing), or to the farmers. It might be argued that Medicare and Medicaid, because of the way they have been administered, have amounted to subsidies to the medical profession. But Medicare and Medicaid are more like food stamps or housing allowances (i.e., relatively minor or even experimental subsidies directly to the consumer in their respective industries) than they are like crop subsidies or mortgage interest subsidies, which go directly to the producer. The federal government does subsidize research and development in health care, at least as extensively as it does in agriculture, or in the military establishment. Largely under the guise of subsidizing research and development, it has also been subsidizing education for health care practitioners, although there are indications that source may be drying up. There are, of course, a variety of state and federal educational subsidies to prepare persons for technical jobs in industry, but probably none as extensive as in health care. It is difficult to separate trends in the incidence of the cost of health care education from trends in the incidence of the cost of professional and technical education generally. But it seems reasonable that as new forms of health care organizations develop, a good deal of that cost may be shifted to them, and through them to the consumer of health care. On the other hand, the creation of larger health care organizations in the private sector may result in new forms of subsidy going directly to the purveyors of health care rather than to the patients. This issue is tangled up with the larger question, as health care becomes less and less a matter of individual enterprise, how far will it move towards consumer control of its new kinds of institutions, and how far towards more traditional producer control. The answer to that question will probably decide who gets the bulk of the subsidies in the future—and how much government regulation goes along with the subsidy.

Technical assistance from government is more likely to be offered to industries with large numbers of very small producers, as in the case of the agricultural extension service; and so long as health care is primarily purveyed by single practitioners, they will continue to rely on a wide range of public technical services, particularly in epidemiology and pharmacology. But as the organization of practice becomes more systems oriented, the technical assistance function may be more and more incorporated into private health care systems which may, in turn, seek to substitute direct governmental subsidies for technical assistance.

The health care industry has had at least one brush with the antimonopoly powers of government, in the litigation over the attempted exclusion of group health physicians from hospital privileges, which seems from our present perspective almost a prehistoric event. That litigation was based on the provision of the law that forbid conspiracies in restraint of trade. It is at least possible that as health care is provided by larger organizations, they may encounter the provisions of the law that have been interpreted to require the breakup (or "divestiture") of organizations that have come to dominate a particular market. This domination may occur in any one or more of a whole range of activities, from primary care, to the provision of laboratory services. It may occur in a national market, where it is subject to federal control, or within a single state, where it may be subject to state antitrust laws, for example, where a state hospital association negotiates with Blue Cross on behalf of all the hospitals in the state.

Health care practitioners are accustomed to regulation at the state and local level. They get their licenses to practice from state officials, and they comply with local rules and ordinances. Health care institutions (including educational institutions) are subject to regulation primarily at the state and local level, and such federal control as they experience is exerted primarily through conditions accompanying grants of federal funds. Individuals and institutions are subject to national certification, by quasi-public bodies. And individuals and institutions experience federal regulation in the production, distribution, and use of drugs.

There is a whole new dimension of regulations developing at the state level. Recognizing that skilled human resources and capital resources are both scarce, government is beginning to restrict the creation or expansion of health care institutions by requiring certificates of need, based on an overall plan for the distribution of health care facilities throughout the state. Under this kind of legislation it is not sufficient that the trustees of X Hospital have raised the funds and found the personnel for their own cobalt bomb or their own open heart surgery unit, and it may not be enough that a group of physicians and nurses and physicans' assistants and other allied health professionals decide to set up shop in a given locality, and put together the space and equipment and working capital to get started. They must still justify their proposed operations on the basis of comparative need for those services in that location.

Two kinds of problems are raised by this developing phenomenon:

1) If we can no longer rely on the market place to distribute health care resources in optimum fashion, then we can no longer assume that the self-interest of consumers and of providers (including providers of health care research and health care education) automatically coincide at the point where cost and demand curves meet. Government, we have discovered, is not an impartial arbiter among competing interests. It matters, therefore, how these competing interests are represented. A certificate of need will not automatically express the Benthamite ideal of the greatest need of the greatest number, and what needs it does express depends on what constituencies—rich, poor, old, young, urban, rural—are represented in the certification process, and how effectively.

2) The second problem is likely to develop as health care becomes more systemized and larger and as more powerful private organizations develop. State-level regulation of strongly organized industries (oil and gas, coal mining, lumbering, or plumbing installation) has characteristically tended to be dominated by industry interests. Experience so far in the Comprehensive Health Planning movement, in spite of the agency efforts to educate consumers, has in most cases shown a similar tendency toward provider control. In order to avoid that domination in health care, federal regulation may become a necessity. Nor can this problem be avoided by channeling the development of new and enlarged health care institutions into the nonprofit area. Nonprofit institutions are at least as likely to respond to internal bureaucratic imperatives for uncontrolled growth as are institutions in the for-profit sector of the economy. A larger staff and a larger budget (whether or not they justify a larger salary or a larger office) are just as much incentives as profit after taxes. If non-profit institutions grow large enough, they can as easily outrun state and local controls as can the most profit-hungry entrepreneurs.

In all its diverse and multifarious roles vis a vis the health of the nation, there are some things where government is distinctly better, and there are some things at which it is distinctly worse. By and large it is better at collecting and paying out money, and it is worse at managing enterprises that involve a high degree of adaptation to individual differences. Government is generally acknowledged to have failed miserably in the provision of social services to the underclass; it is generally thought to have succeeded brilliantly in the administration of direct payments schemes under the Social Security program. Apart from its responsibilities for public safety and national defense (areas in which it has scarcely been distinguishing itself lately), and apart from its direct operations in primary and secondary education, government is essentially concerned with the redistribution of resources that are not effectively (or equitably) distributed in the market place. These resources can be redistributed, directly or indirectly, in a number of ways: by collecting taxes, by setting minimum standards, by offering subsidies, by offering services, and by giving various forms of political encouragement to would-be redistributors (and corresponding discouragement to those who would preserve an undesirable status quo or pursue other disfavored distribution schemes).

To the officers of government, both legislative and executive, on whom all the pressures of the citizenry impinge, health care does not appear as a well-distributed resource—certainly worse distributed than food or clothing, or transportation, probably worse distributed than shelter, or education. They recognize that it is an extraordinarily difficult resource to produce and to manage. But by the same token, they are coming to realize that health care is a system, from preventive and primary care through secondary and tertiary care, long-term rehabilitation and chronic care, and that what is required at each stage is not an individual provider but an institution, or a range of institutions, each of which must mesh with all the other institutions in the system.

Both politicians and bureaucrats are extremely reluctant to intervene in the health care system. They recognize the very high degree of expertise of most health care professionals, and most of them probably still see the physician, even through the haze of recent years of adverse publicity, as a kind of father figure. But they also recognize that the system requires an equally high degree of managerial skill in order to make it function at an even minimal level of effectiveness, and that without those skills the system will either fail to meet public expectations—and government will get most of the blame. Unless there is evidence that those managerial skills will be supplied from within the system—or by private citizen initiative—then government will feel obliged to supply them.

But even if, and particularly if, management skills are supplied from within the health care system, government will still feel an obligation to shape the distribution of health care resources in accordance with the relative strengths of the various forces outside the system. At the most practical level, government will feel this obligation because it will feel the pressure of the various interested constituencies, to the extent they are not adequately represented within the system. On the level of political theory, government will feel this obligation because that is a central function of government, to the extent it is not adequately performed by private agreement among all the interested parties. There is no theoretical optimum division of health care resources between reducing neonatal mortality, and improving the physical condition and the morale of stroke victims; between extending the availability of renal dialysis and of psychotherapy; between basic research on cell growth and training of ambulance drivers in the use of defibrillators.

If to govern is to choose, then making the hardest choices as they affect the shape of the health care system is increasingly the responsibility of government. Beyond the rhetoric of a universal right to health care (or the extraordinary claim of a universal right to health) government is accountable for the hard decisions on what resources go into the health care system and how health care is distributed. On the first question, government must and will make the final decisions. On the second question, government must and will satisfy itself that health care consumers are fully and fairly represented in allocating health care. If they are not, government cannot avoid stepping in to take over the allocation process itself. The political man, as well as the medical man, must make health care decisions that are literally matters of life and death.

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# The Implications of Hyperammonemia in Rare and Common Disorders, including Migraine

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## *I. INTRODUCTION*

Awareness of the existence of hereditary ammonia intoxication with underlying enzymatic defect of the urea cycle, or of its grave neuropathic import in infancy or childhood, or even of the essentials of its specific clinical expression in these age phases dates back only ten years to our definition of congenital hyperammonemia. We were also able to reveal a deficiency or defect of the enzyme ornithine transcarbamylase (OTC) which normally catalyzes the conversion of carbamyl phosphate and ornithine to citrulline (Fig. 1). This first exposure of an inborn enzymatic defect within the Krebs-Henseleit urea cycle itself and, paradoxically enough, in a condition in which urea output was believed to be so normal, also helped eventually to clarify the basic pathogenesis of argininosuccinicaciduria (ASA), which had been reported as a new aminoaciduria four years before. Allegedly normal blood urea levels in this disorder had then been interpreted as precluding any possibility of a primary enzymatic block within the Krebs-Henseleit urea cycle. Likewise, when my colleagues Levin, Mackay, and Oberholzer recorded the second ASA family in 1961, the possibility of accumulation of ammonia excess was once again neither sought nor mentioned; and the sinister "nitrogen skeleton in the cupboard"—ammonia intoxication—continued to dangle unseen.

In August 1961, I was called to the Westminster Children's Hospital to see J.M. (Fig. 2), then twenty months old. A third surgical intervention had been seriously mooted in a despairing effort to stem the worsening episodes of profound vomiting and collapse which had been recurring from the first month of life. Her gross developmental failure, with even nuchal control still to be attained, and a head circumference of 16.5 inches helped to orient the case in a nonsurgical direction. Analysis of these vomiting episodes etched in predictable clinical sequence what I believe to be the classic clinical expression of hyperammonemia in a child (Fig. 3). After a step-by-step appearance and accentuation of malaise, pallor, anorexia, lassitude, apathy, and nausea, vomiting would begin—sometimes explosively, sometimes with blood—and steadily intensify in parallel with intermittent agitation and screaming, sometimes accompanied by

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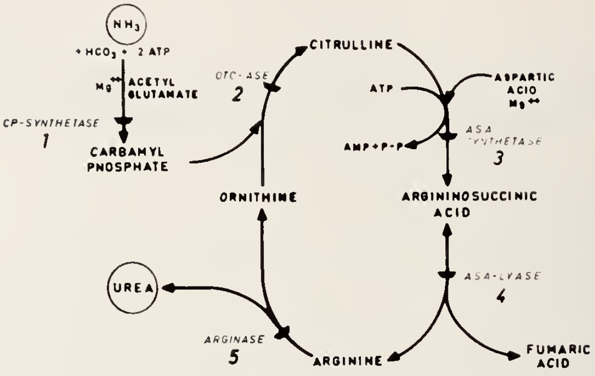
ORNITHINE-UREA CYCLE OF KREBS-HENSELEIT
*CATALYZED BY FIVE ENZYMES*



| ENZYME DEFECTS AT EACH STEP | DEFINITION OF UNDERLYING HYPER NH$_3$ |
|---|---|
| 1. *HYPERAMMONAEMIA TYPE I CPS*<br>*CARBAMYLPHOSPHATE SYNTHETASE* | FREEMAN ET AL 1964 |
| 2. *HYPERAMMONAEMIA TYPE II OTC*<br>*ORNITHINE TRANSCARBAMYLASE* | RUSSELL ET AL 1962 |
| 3. *CITRULLINAEMIA*<br>*ASA SYNTHETASE* | McMURRAY ET AL 1963 |
| 4. *ARGININE SUCCINIC ACIDURIA*<br>*ASA LYASE* | RUSSELL ET AL 1963-66<br>EFRON ET AL 1967 |
| 5. *ARGININAEMIA*<br>*ARGINASE* | TERHEGGEN ET AL 1969 |

FIG. 1. Krebs-Henseleit Ornithine-Urea Cycle: Illustrating sites of the known inborn defects of its five enzymatic sequences, and the timing of definition of their underlying hyperammonemia.

repetitive hand movements to the forehead as if in response to excruciating headache. A slowly deepening lethargy culminated in what was superficially regarded as collapse.

The history of J.M.'s six-year-old first cousin revealed analogous features. The cousin, one of presumptive identical twins, was at that time awaiting discharge from the National Hospital for Nervous Diseases, Queen's Square, with a confident diagnosis of "ophthalmoplegic migraine." Onset in this case was deferred until her fourth year. Since then there had been recurrent episodes of vomiting bound up with headache, visual aura including fortification spectra and diplopia, crying, screaming hallucinations, and transient bilateral ptosis. But, a more sinister element tending to impel her beyond the pale of a usual migraine was our elucidation of gradual intellectual regression, diffusely pathologic electroencephalography, and deceleration of cranial and skeletal growth relative to her twin who served as a species of control but was herself subsequently shown to be heterozygous for the trait.

What prompted those first estimations of blood ammonia? In essence, a succession of observations built up to complete the basic pattern in this challenging jigsaw. During a hydrogen ion clearance test undertaken to exclude renal tubular acidosis as a basis for the persisting alkalinity of urinary pH, 98 percent of the total hydrogen ion excreted proved to be ammonium ion. This

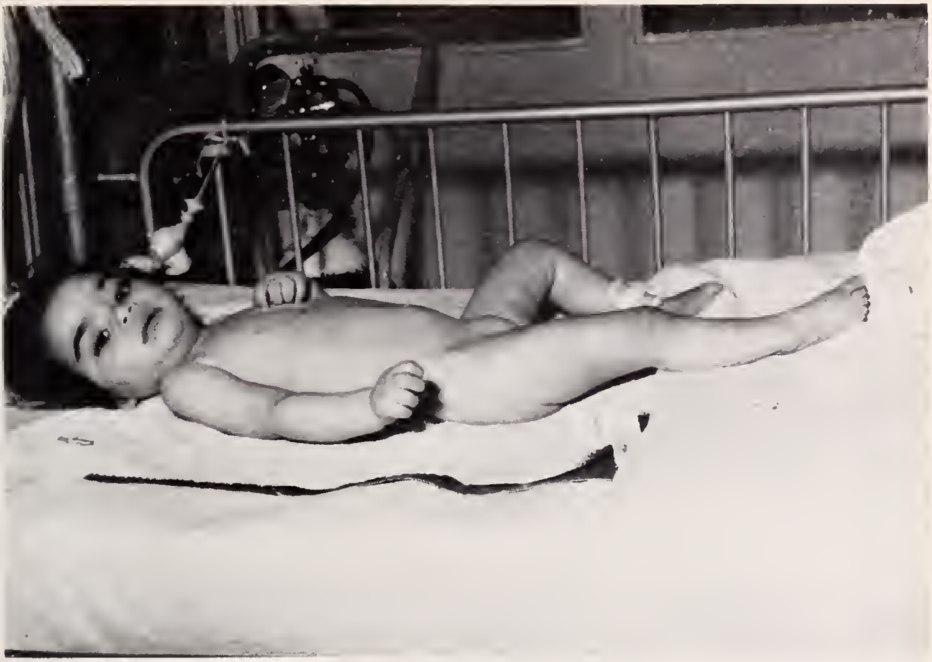


FIG. 2. Case 1 (J.M.) at 16 months: First example of inborn OTC-ase defect with failure of cranial growth, cerebral degeneration, and developmental retardation, dating back to the sixth month.

figure far exceeded levels appropriate to the distal-segment type of renal tubular acidosis (Fig. 4) which would be marked by a consistently high urine pH.

Then the hospital staff soon confronted me with her abruptly renewed collapse and the need to explain its emergence earlier than its usual three-to-four-week cycle. Moreover, it clearly swept upon her within 36 hours of the test. That initial result implied the possibility of underlying excess of blood as well as urinary ammonia. If so, the ammonium salt load could well have been the precipitating factor in the sudden worsening of the child's condition.

It was this iatrogenic episode which provided the first opportunity to identify the evolution and fluctuation of several clinical components. The whole clinical pattern of the attack required reappraisal. Instead of the "collapse" featured in earlier case notes, rousability and stupor became apparent as the infant responded to intrathecal instillation of saline, which was used because of profoundly low CSF pressure found in association with my observation of extreme retinal vascular constriction or depletion. The premonitory clinical sequence had thus really culminated in stupor, deepening only intermittently into coma. Added features included hyperreflexia, tonic seizures, spontaneous ankle clonus, and a curious repetitive hand-waving. My sudden appreciation of the latter as the possible infantile counterpart of adult "hand flaps" helped to complete an impression that we might be witnessing the cumulative neuropathic phases which have been more clearly identified during the progression of liver failure in the adult (Fig. 5a and b).

Another major clue was the awakening to the significance of transiently raised urinary glutamine in the patients on two occasions. Could it have reflected maximal activity of so crucial a mechanism of ammonia binding as the alphaketoglutarate-glutamic acid-glutamine system (Fig. 6)—its apparent in-

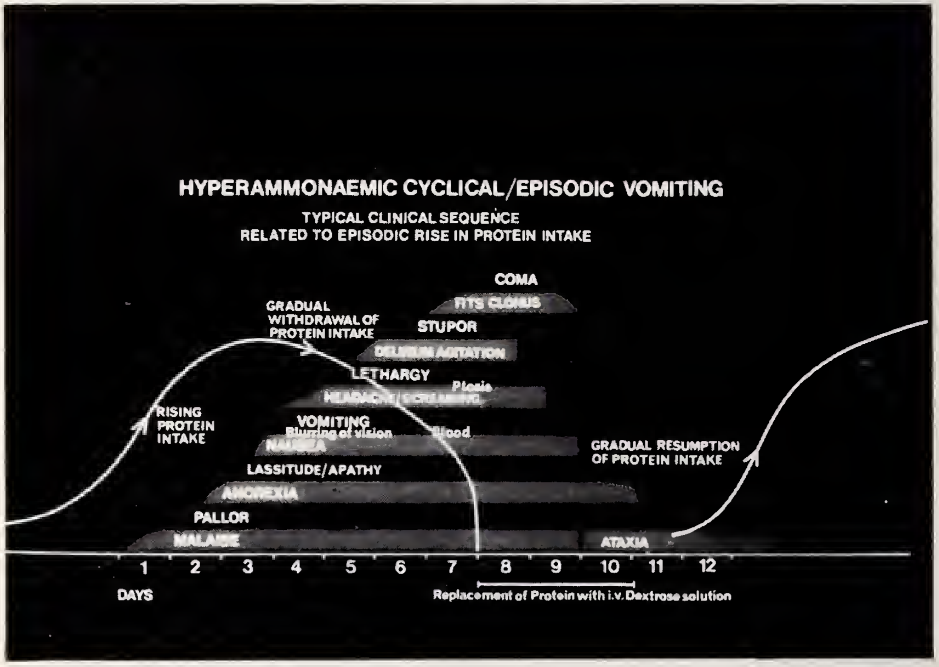

HYPERAMMONAEMIC CYCLICAL/EPISODIC VOMITING
TYPICAL CLINICAL SEQUENCE
RELATED TO EPISODIC RISE IN PROTEIN INTAKE
COMA
FITS CLONUS
GRADUAL WITHDRAWAL OF PROTEIN INTAKE
STUPOR
DELIRIUM AGITATION
LETHARGY
Ptosis
HEADACHE/SCREAMING
RISING PROTEIN INTAKE
VOMITING
Blurring of vision
Blood
NAUSEA
GRADUAL RESUMPTION OF PROTEIN INTAKE
LASSITUDE/APATHY
ANOREXIA
PALLOR
MALAISE
ATAXIA
1
2
3
4
5
6
7
8
9
10
11
12
DAYS
Replacement of Protein with i.v. Dextrose solution

FIG. 3. Core of clinical expression of hyperammonemia in infant or child: Cumulative step-wise progression and intensification of its components. Cardinal treads link lassitude to stupor and coma. Relationship to recurring increase or resumption of protein intake indicates basis for so-called "cyclicity" iatrogenically induced.

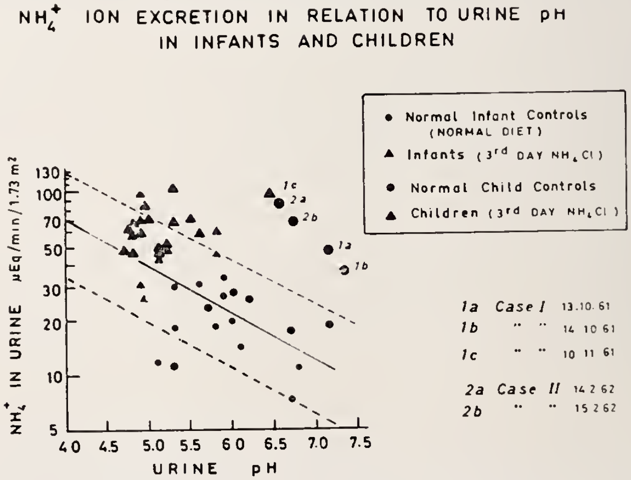
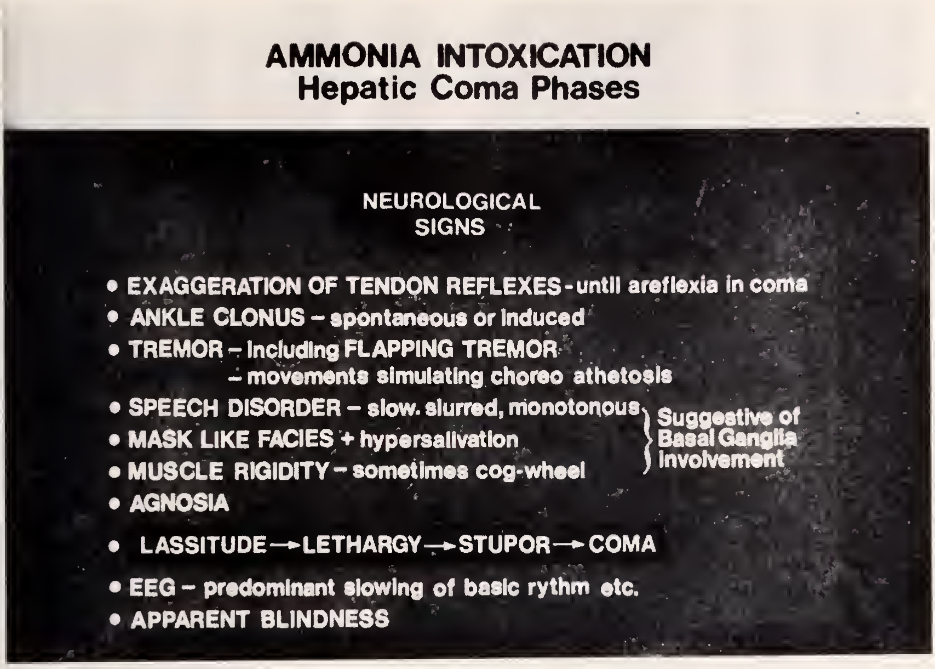


FIG. 4. Note the exceptionally high proportions of H-ions excreted as $NH_4^+$ both in the infant (Case 1) and the elder child (Case 2) homozygous for OTC-ase defect.



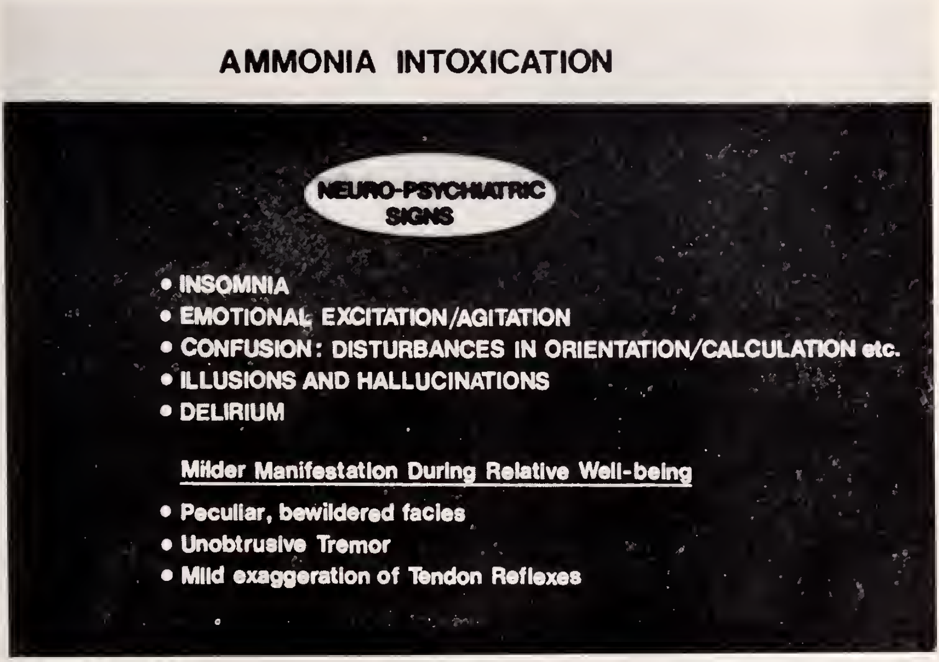
**AMMONIA INTOXICATION**
**Hepatic Coma Phases**

NEUROLOGICAL
SIGNS

- EXAGGERATION OF TENDON REFLEXES - until areflexia in coma
- ANKLE CLONUS – spontaneous or induced
- TREMOR – including FLAPPING TREMOR
  - – movements simulating choreo athetosis
- SPEECH DISORDER – slow. slurred, monotonous
- MASK LIKE FACIES + hypersalivation
- MUSCLE RIGIDITY – sometimes cog-wheel

} Suggestive of Basal Ganglia involvement

- AGNOSIA
- LASSITUDE → LETHARGY → STUPOR → COMA
- EEG – predominant slowing of basic rythm etc.
- APPARENT BLINDNESS



**AMMONIA INTOXICATION**

NEURO-PSYCHIATRIC
SIGNS

- INSOMNIA
- EMOTIONAL EXCITATION/AGITATION
- CONFUSION: DISTURBANCES IN ORIENTATION/CALCULATION etc.
- ILLUSIONS AND HALLUCINATIONS
- DELIRIUM

Milder Manifestation During Relative Well-being

- Peculiar, bewildered facies
- Unobtrusive Tremor
- Mild exaggeration of Tendon Reflexes

FIG. 5a and b. Cumulative neuropathic components characterizing hepatic failure in the adult (upper and lower sections).

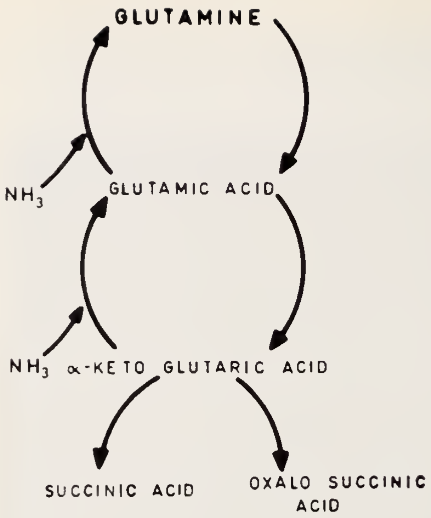


FIG. 6. A crucial mechanism of ammonia binding: the alphaketoglutarate-glutamic acid-glutamine complex.

constancy would have meant little more than that the relevant urinary collection had not coincided with the peak of clinical crisis.

Retrospectively, one could also recognize that the resolution of each of these crises had coincided with the substitution of intravenous dextrose solutions for oral protein-feeding.

All these facts donned doubly enhanced significance when we oriented their interpretation in accord with hepatic stupor in reflection of episodic ammonia intoxication, or a state comparable to "episodic hepatargy" in the human with ECK fistula, or to the "meat intoxication syndrome" of the ECK fistula dog. It is also true, however, that at first sight these inferences did appear flimsy in the perspective of a normal array of liver-function tests. The interesting exception of strikingly high levels of serum transaminase activity (both of SGOT and SGPT) we shall discuss later.

Nonetheless, we soon put our speculations to test and with the assistance of Sheila Sherlock, in the only laboratory in London at that time routinely undertaking blood ammonia estimations, we were soon able to demonstrate highly pathologic excesses of ammonia both in the CSF and the blood of both children. Furthermore, it was challenging to realize that in each compartment CSF, blood, and urine, elevations of glutamine and of pH paralleled those of ammonia.

An underlying inborn deficiency or defect of one of the Krebs urea cycle enzymes was our postulate and, in the absence of urinary excess of citrulline, argininosuccinic acid, or arginine, a defect had to be within the first two enzymatic steps of the cycle. While my colleagues Oberholzer and Levin prepared the assay methodology in liver biopsy material of Krebs urea cycle enzymes, exclusion at laparotomy of the remote possibility of some congenital portocaval anomaly was also planned. We went on to firmly establish the critical deficiency of ornithine transcarbamylase (OTC) activity already anticipated. Its activity was no more than five percent of normal in Case 1 and in the older cousin, with belated onset of the disorder, it was demonstrated to be about 15 percent. Later, such severely reduced OTC activity was also confirmed in the intestinal mucosa of Case 1.

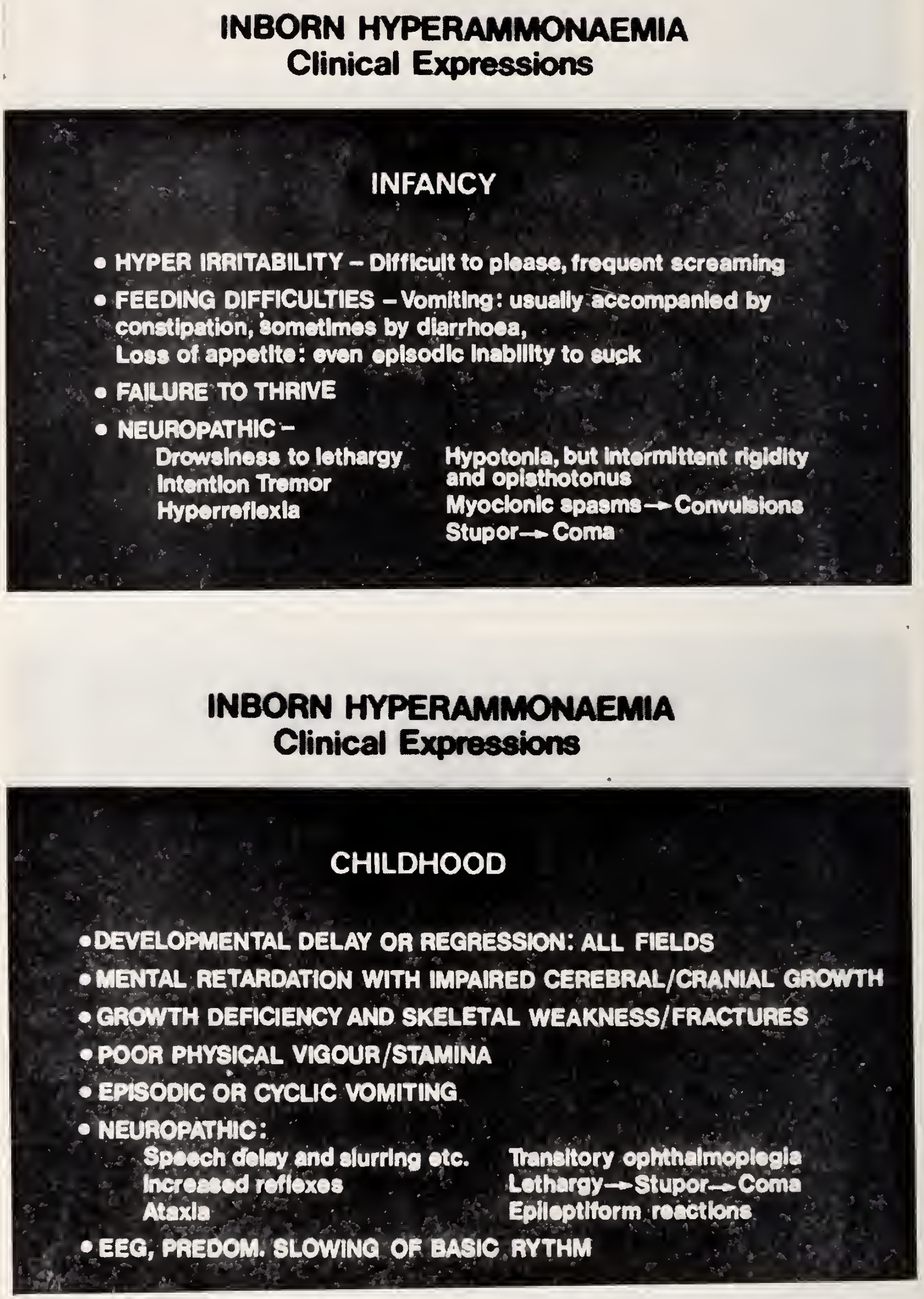
## *II. RECOGNITION OF HYPERAMMONEMIA AND ITS ROLE IN THE ELUCIDATION OF FURTHER ENZYMATIC DEFECTS OF THE KREBS UREA CYCLE*

Historically, therefore, this first child and her cousin were of fundamental importance as prototypes of a whole family of disorders yet to emerge, each mutually marked as a member of the family by an underlying hyperammonemia. It was the clinical pattern which proved to be sufficiently specific to suggest the ammonia intoxication and thence to point the way to the Krebs urea cycle as the template of operation of its enzymatic defect.

To comprehend the specificity of this clinical pattern and the implication of each evolving component, it behooves us to cultivate some familiarity with at least the clinical expressions of hepatic coma or of its experimental analogues. It is important to think in terms of progressive phases of a graduated intensification of clinical or experimental hepatic coma, or rather as the evolving expression of an increasing concentration of underlying hyperammonemia (Fig. 7a and b). One must keep in mind, in addition, the possibility of superimposed effects stemming from related biochemical factors, disturbances of which can so closely simulate, or be secondary to, the influence of excessive ammonium ion. Particularly in this context, we draw attention to the alkalosis present in both our initial OTC cases. Their high urinary pH was associated with a blood pH of 7.49 and 7.48 respectively, one CSF pH spontaneously reaching 7.59 during a crisis.

The respiratory alkalosis in adult liver disease is attributed to a direct stimulatory influence of the ammonia excess upon the respiratory center. It is the proportion of *nonionized* ammonia which increases in these circumstances, and in this form it is believed to penetrate more readily through the cell membrane. Pursuing the diffusion theory of Milne, Scribner, and Crawford, accumulation of the nonionized ammonia should be on the less alkaline side of a semipermeable membrane. Ammonia diffusion would thus be towards the intracellular side when alkalemia prevails in the presence of hyperammonemia. The suggestion has followed that it is probably the partial pressure of ammonia ($pNH_3$) which is the relevant factor in producing, or enhancing, its toxicity.

Anemia or hypokalemia have also been found to predispose to heightened sensitivity to ammonia. Even magnesium deficiency appears to contribute to enhanced $NH_3$ toxicity. This latter mineral could well be of special significance in this context since it constitutes a cofactor vital to at least two enzymatic stages of the urea cycle itself. That the characteristic tremor and exaggerated reflexes could in part reflect a specific magnesium deficiency, whether primary or secondary, is supported by the partial improvement which may follow intramuscular magnesium sulphate.



## INBORN HYPERAMMONAEMIA
## Clinical Expressions

### INFANCY

- HYPER IRRITABILITY – Difficult to please, frequent screaming
- FEEDING DIFFICULTIES – Vomiting: usually accompanied by constipation, sometimes by diarrhoea,
  Loss of appetite: even episodic inability to suck
- FAILURE TO THRIVE
- NEUROPATHIC –
  - Drowsiness to lethargy
  - Intention Tremor
  - Hyperreflexia
  - Hypotonia, but intermittent rigidity and opisthotonus
  - Myoclonic spasms → Convulsions
  - Stupor → Coma

## INBORN HYPERAMMONAEMIA
## Clinical Expressions

### CHILDHOOD

- DEVELOPMENTAL DELAY OR REGRESSION: ALL FIELDS
- MENTAL RETARDATION WITH IMPAIRED CEREBRAL/CRANIAL GROWTH
- GROWTH DEFICIENCY AND SKELETAL WEAKNESS/FRACTURES
- POOR PHYSICAL VIGOUR/STAMINA
- EPISODIC OR CYCLIC VOMITING
- NEUROPATHIC:
  - Speech delay and slurring etc.
  - Increased reflexes
  - Ataxia
  - Transitory ophthalmoplegia
  - Lethargy → Stupor → Coma
  - Epileptiform reactions
- EEG, PREDOM. SLOWING OF BASIC RYTHM

Fig. 7a and b. Clinical expressions of inborn hyperammonemia in infancy and childhood (upper and lower sections).

## *III. CLINICAL PATTERN AND KINSHIP TO EPISODIC HEPATIC STUPOR*

While recurring episodes of vomiting linked to failure to thrive was the essential presenting pattern in our OTC Case 1 from the age of five weeks, screaming episodes and behavior disorder clearly preceded the onset of episodic vomiting in OTC Case 2 during her fourth year. But, once established, the vomiting became no more than one facet of a predictable course through several overlapping clinical stages progressing to stupor and coma. Suspicion of underlying hyperammonemia in our own cases was finally prompted by recognition of an episodic pattern of symptomatology rapidly dominated by lethargy to stupor, and other analogies with these progressive neuropathic sequences.

A rough clinical grading of these sequences has some applicability to our case, the rapidity of advance varying from hours to days, although usually most precipitant in the more severe neonatal or infantile cases, especially Types I and II of hyperammonemia or of ASA. The first stages of each episode are marked by malaise, apathy, lack of awareness and alertness, or by some impairment of intellect or comprehension, with or without objective neurologic signs. Increasing anorexia was also troublesome in our children, manifest in gagging or evident nausea when feeding was forced. Lassitude and muscle weakness also insidiously worsened, and slurring of speech was often already marked at this stage in Case 2.

The second stage of predominant personality change was characterized in our children by restlessness, psychomotor agitation, temper tantrums, and bizarre behavior, as in the elder child, alternating in both with advancing lethargy. Nausea had by then given way to repetitive vomiting, occasionally with minor hematemesis. Neurologic signs at this stage included apparently intense unilateral headache, in both cases marked by screaming—in Case 1 also by opisthotonus and repetitive hand movement to the forehead, and in Case 2 even by head-banging or tearing at hair and eyes. Visual aberrations, blurring of vision, diplopia, or even apparent amaurosis was identifiable in the elder cousin. The characteristic expressionless or mask-like facies was often evident, although frequently interrupted by bizarre grimacing. Ptosis then usually appeared in the elder child, and although she might display some staggering at this stage, muscle weakness was the major factor, true ataxia being more typical of her "recovery" phase.

The next stage of frank confusion and disorientation was often scarcely separable from the foregoing features; sometimes it progressed to a wild delirium as in Case 2, in which underlying hallucinations clearly played a role.

As their lethargy mounted to the next stage of stupor, the more specific neurologic criteria were likely to have become established: increasing hypertonus or actual paratonic rigidity of cogwheel type; bilateral knee and ankle clonus, the latter often spontaneous and sometimes sustained; hyperreflexia and the bilateral Babinski, although often unequal or fluctuant. We regarded the peculiar hand-waving movements as the possible counterparts of the hand-flaps of adult liver failure. Hyperpnea was typical in this phase. Intermittent to-and-fro transition into the final stage of coma marked the first case; breathing often became periodic and of Cheyne-Stokes form. Occasionally, this stage was immediately preceded or punctuated by muscle-twitching or full convulsions.

## *IV. GENETIC SIGNIFICANCE*

The mode of inheritance still remains unclear. On the basis of the known data, OTC deficiency would appear to be sex-limited, at least in its expression. This considers the facts that in our pedigree both twins and their first cousin were affected; that at least one mother showed 50 percent OTC deficiency; that six of seven cases have been female (one male representing an enzyme variant with different substrate affinity and pH optima); and that investigation of three of the fathers proved negative. However, Campbell et al have recently been able to document a lethal male neonatal example who succumbed to extreme hyperammonemia on his fifth day of life, to reveal a uniquely complete absence of hepatic OTC activity. They postulate that OTC is coded for by a gene on the X-chromosome, mutations of which can lead to partial enzyme deficiency in heterozygous females due to disadvantageous Lyonization, and to total absence of the enzyme in hemizygous males.

## *V. OTHER INBORN DEFECTS OF THE KREBS UREA CYCLE AND RELATED PATHWAYS*

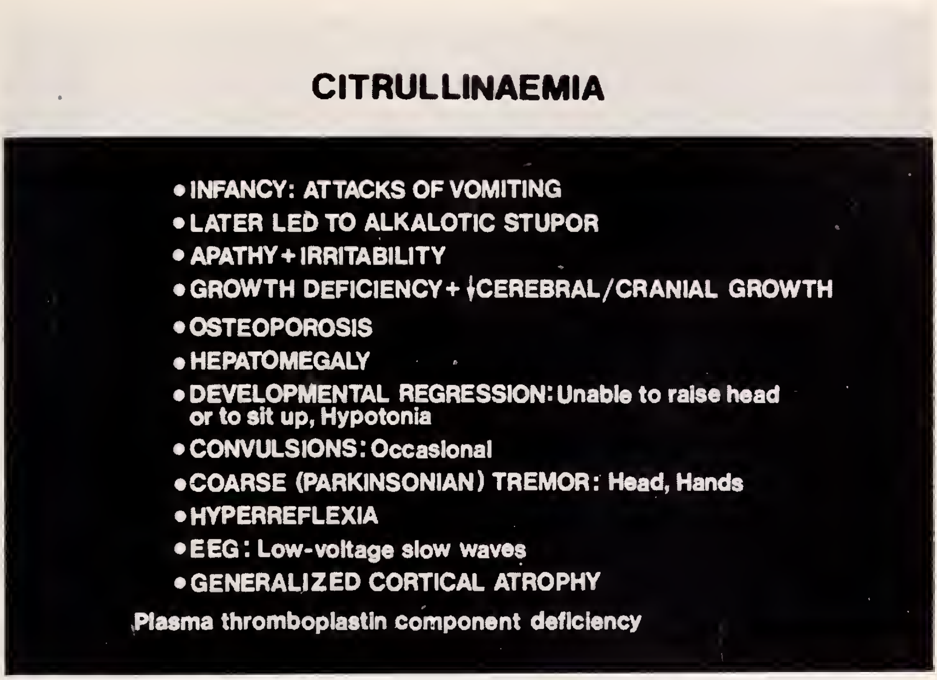
### *A. Citrullinemia*

In the same year as our own report, this disease was introduced by McMurray et al as "citrullinuria," ostensibly just another amino acid disorder associated with mental retardation. Despite normal levels of blood urea, an enzymatic defect at the third step of the Krebs cycle (ASA synthetase) had not yet been sought, nor had the possibility of underlying hyperammonemia. The clinical description of this syndrome is summarized in Figure 8.

Lowering of the protein intake could eliminate the specific amino acid excess, but it was almost a year before the concomitant blood ammonia was investigated and reported, presumably in light of the hyperammonemia we had exposed as an expression of inborn enzymatic defects of the Krebs urea cycle. It had become clear that blood ammonia was driven up by protein loading as dramatically as it may mount in OTC deficiency—literally, to over 100 $\mu$g/100 ml. Later, Tedesco and Mellman were able to demonstrate, in fibroblast cultures from a citrullinemic patient, that an inactive mutant enzyme of ASA-synthetase appeared to be responsible.

### *B. Carbamyl Phosphate Synthetase Defect*

Carbamyl phosphate synthetase catalyzes the first step of the urea cycle, combining ammonia from N-acetyl glutamate with carbon dioxide in the presence of ATP to form carbamyl phosphate. During our enzyme assays of the initial hyperammonemic cases, a moderate reduction of CP synthetase was also



CITRULLINAEMIA

- INFANCY: ATTACKS OF VOMITING
- LATER LED TO ALKALOTIC STUPOR
- APATHY + IRRITABILITY
- GROWTH DEFICIENCY + ↓CEREBRAL/CRANIAL GROWTH
- OSTEOPOROSIS
- HEPATOMEGALY
- DEVELOPMENTAL REGRESSION: Unable to raise head or to sit up, Hypotonia
- CONVULSIONS: Occasional
- COARSE (PARKINSONIAN) TREMOR: Head, Hands
- HYPERREFLEXIA
- EEG: Low-voltage slow waves
- GENERALIZED CORTICAL ATROPHY

Plasma thromboplastin component deficiency

Fig. 8. Citrullinemia: cardinal clinical features.

detected and, indeed, this appears to have been the rule for subsequent examples of inborn OTC defect.

In January 1964, Dr. Masland wrote to me concerning a 10-week-old infant whose recurrent episodes since ten days of age of intense vomiting, hypotonia, fits, and lethargy to stupor or coma were precipitated by a casein hydrolysate formula (Nutramigen®). This clinical pattern had been shrewdly recognized as reminiscent of our first case. Brief neonatal hyperbilirubinemia was in the background and had also marked the story of an immediately preceding sibling who had died at five months of age after recurrent attacks of vomiting, lethargy, and coma, together with persistent acidosis and intermittent neutropenia, ending in convulsions and both blindness and deafness.

Increased plasma glutamine levels were associated with the triad of mild hyperglycinemia (3.4 to 5.1 mg/100 ml), mild metabolic acidosis, and cyclic neutropenia. This was also found to typify the ketotic hyperglycinemia described with hyperammonemia by Soriano et al.

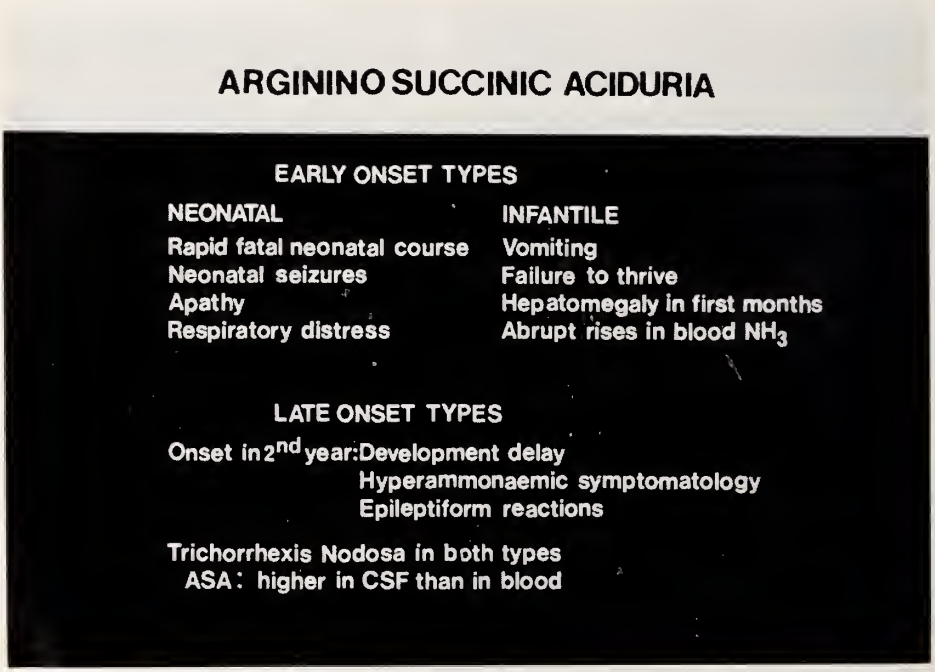
Restoration to clinical and biochemical normality after protein restriction (to 0.8–1.0 gm/Kg/day) was claimed in this letter, but the patient nonetheless failed to gain weight, developed hypoalbuminemia and osteoporosis, to succumb at five months of age—precisely as the sister already mentioned succumbed.

This time, a more substantial reduction of CPS activity (to 22%) was clearly defined in liver biopsy material. This low level of activity may, of course, reflect an altered enzyme with different affinities and kinetics, rather than impaired synthesis of a normal enzyme, but it clearly involves the CP synthetase which is concerned with the synthesis of urea rather than that of pyrimidine. The in vitro reduction of CP synthetase suggests that at least this was the site of the defect in vivo. It is possible, nevertheless, that such inborn primary defects may lie even before CP synthetase, within the ammonia transport or ATP delivery system.

### *C. Argininosuccinic Aciduria*

To date, at least 26 examples have been recorded in varying degrees of detail. The clinical pattern of presentation can be divided, according to the age of onset, into two main categories: "early" and "late" (Fig. 9). Of the former, of which there have been eleven examples, a group marked by neonatal presentation is the most calamitous and lethal, with vomiting and apathy, advancing to progressive somnolence and coma, convulsions, respiratory distress with underlying pulmonary hemorrhage, and gross hepatomegaly. In one such affected family running true to type, four siblings died of this so called "malignant" form within days of birth, while in another family three siblings died on the fourth day of life in extreme hyperammonemia, a blood ammonia of 850 μg/100 ml being recorded in the third sibling.

In the late-onset type, there may have been a history of hyperirritability and feeding difficulties in early infancy, but the classic ASA symptom complex of



ARGININO SUCCINIC ACIDURIA

EARLY ONSET TYPES

| NEONATAL | INFANTILE |
|---|---|
| Rapid fatal neonatal course | Vomiting |
| Neonatal seizures | Failure to thrive |
| Apathy | Hepatomegaly in first months |
| Respiratory distress | Abrupt rises in blood $NH_3$ |

LATE ONSET TYPES

Onset in 2nd year: Development delay
Hyperammonaemic symptomatology
Epileptiform reactions

Trichorrhexis Nodosa in both types
ASA: higher in CSF than in blood

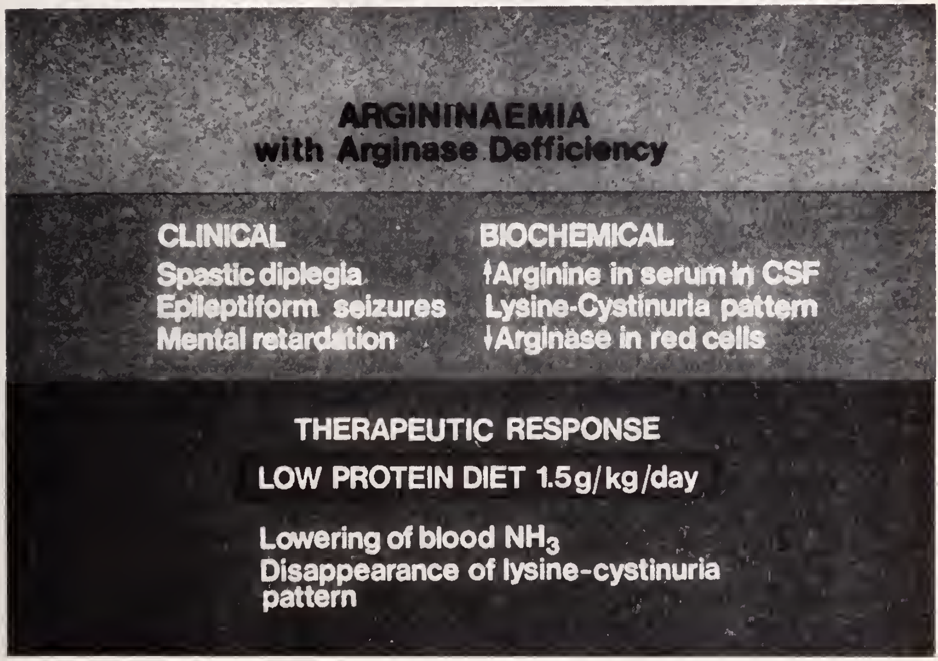
FIG. 9. ASA: Cardinal clinical pattern according to age phase (so-called "early" and "late" onset types).

mental retardation, seizures, intermittent ataxia, diminished cranial growth, and hepatic dysfunction (marked, for example, by high plasma alkaline phosphatase levels) evolves slowly. Neuropathic features predominate at this stage: speech development may appear disproportionately immature, although on the whole mental retardation is uniform; the unsteadiness of gait and of hand movement is typical, but may fluctuate in intensity especially worsening after a seizure.

### *D. Hyperargininemia (Fig. 10)*

At last, in 1969, the inborn enzymatic errors of the fifth and last step in the urea cycle were unearthed by virtue of another aminoaciduria, this time of arginine. Two sisters, 18 months and five years of age, presented a triad of mental retardation, epileptic seizures, and spastic diplegia. This was linked to hyperammonemia in association with low or absent arginase activity, and an accumulation of arginine in blood, urine, and CSF. Arginase, as measured in erythrocytes, and not in liver, demonstrates 120 μmoles per gram of hemoglobin in one sister, zero in the other, the normal range being 793 to 1330. Of special interest in an example of inborn urea cycle disorder is that, for the first time, a father showed reduction of relevant enzyme activity (the mother was also affected, but to a lesser extent).

In a number of other disorders, also apparently heredofamilial, elevated blood ammonia levels have been successfully sought on the basis of a similar



**ARGININAEMIA**
**with Arginase Defficiency**

**CLINICAL**
Spastic diplegia
Epileptiform seizures
Mental retardation

**BIOCHEMICAL**
↑Arginine in serum in CSF
Lysine-Cystinuria pattern
↓Arginase in red cells

**THERAPEUTIC RESPONSE**
LOW PROTEIN DIET 1.5g/kg/day

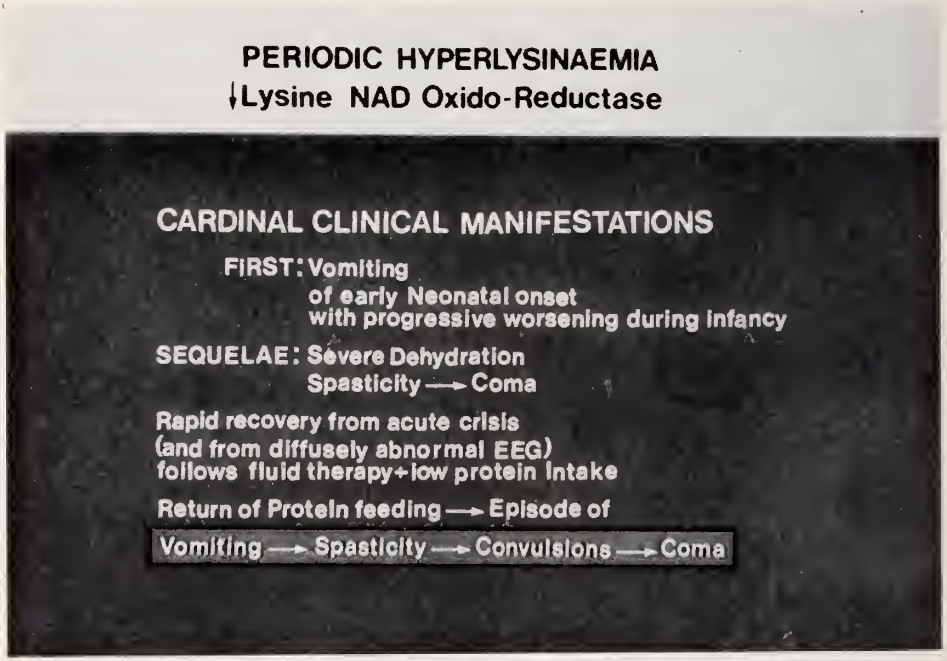
Lowering of blood $NH_3$
Disappearance of lysine-cystinuria pattern

Fig. 10. Argininemia: Principal parameters, clinical and biochemical.

small complex of clinical features originally characterized in congenital hyperammonemia.

### E. *Periodic Hyperlysinemia* (*Fig. 11*)

Periodic exacerbations of hyperlysinemia without hyperlysinuria characterize this defect, and appear to bring about hyperammonemia through competitive inhibition of arginase, perhaps in brain as well as in liver. This enzyme has about 1.6 times greater affinity for lysine than it has for arginine itself. Strong support for this hypothesis derives mainly from two observations: firstly, the coexisting high plasma level of arginine indicating failure of its conversion; and secondly, the direct measure of arginase activity in red cells during lysine loading when blood ammonia rose to 680 μg/100 ml, revealed a parallel decrease in the arginase activity, falling from 227 to 54 μmoles hr/gm Hb at 3 hours, and then to "no activity" at 4 hours, accompanied by coma in the patient! The EEG showed diffuse abnormality and low voltage with high waves over the right hemisphere. Episodes of vomiting, diarrhea, spasticity, convulsions, and coma recurred in tune with blood $NH_3$ concentrations ranging between 500 and 600 μg/100 ml, and a CSF ammonia reaching 165 μg/100 ml, while the urea-N was never more than 7.6 mg/100 ml. The first clinical manifestations of the deterioration, which may closely follow weaning, may be attributed to the higher lysine content of cow's milk. Reduction to 1.5 gm/Kg/day will lead to improvement in the general condition.



**PERIODIC HYPERLYSINAEMIA**
**$\downarrow$Lysine NAD Oxido-Reductase**

**CARDINAL CLINICAL MANIFESTATIONS**

FIRST: Vomiting
of early Neonatal onset
with progressive worsening during infancy

SEQUELAE: Severe Dehydration
Spasticity → Coma

Rapid recovery from acute crisis
(and from diffusely abnormal EEG)
follows fluid therapy+low protein intake

Return of Protein feeding → Episode of
Vomiting → Spasticity → Convulsions → Coma

FIG. 11. Periodic Hyperlysinemia.

What of the origin of the primary hyperlysinemia itself? A partial defect in the first step in lysine catabolism is suspect, or to be more precise a partial defect in the oxidative deamination of lysine stemming from a reduction to 20 to 25 percent of normal activity of L-lysine NAD oxido-reductase.

In a more persistent form of hyperlysinemia with the plasma lysine about four to five times normal (or rather, ranging between 22.5 to 24 $\mu$g/100 ml) Ghadimi et al (1965) initially claimed that only moderate elevation of blood $NH_3$ marked clinical crises precipitated by lysine loading. Principally, these were comprised of abdominal pain, vomiting, ptyalism, muscle weakness, and lethargy. In four of the seven recorded examples, although learning to walk was accomplished before two years of age, gradations of mental retardations were to remain.

### *F. Hyperornithinemia (Fig. 13)*

In one of the two forms of hyperornithinemia so far described, that of Shih et al (1969), hyperammonemia has been substantiated. Neither specific defect in ornithine ketoacid transaminase nor of other relevant enzymes which could interfere with the subsidiary pathway from ornithine to glutamic acid semialdehyde and proline has yet been identified (Fig. 12) in contrast to this finding in the so-called nonhyperammonemic form presented by Bickel and his colleagues.

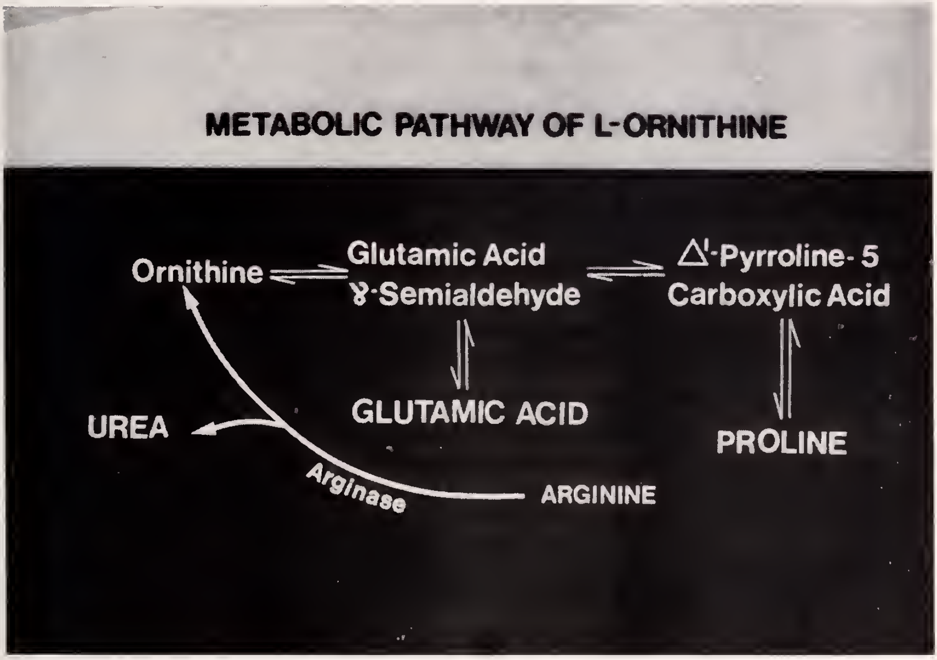
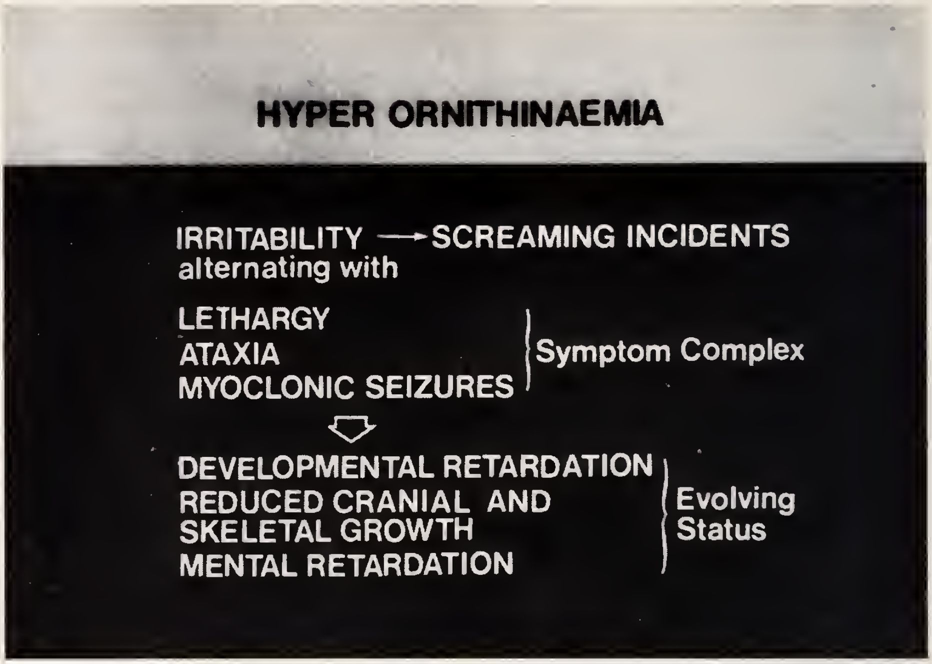


FIG. 12. Segment crucial to Krebs urea cycle, ending in urea and ornithine, and subsidiary degradative pathway from ornithine to proline, wherein enzymatic or other aberration may account for hyperornithinemia.



**HYPER ORNITHINAEMIA**

IRRITABILITY → SCREAMING INCIDENTS
alternating with

LETHARGY
ATAXIA
MYOCLONIC SEIZURES
} Symptom Complex

⇩

DEVELOPMENTAL RETARDATION
REDUCED CRANIAL AND SKELETAL GROWTH
MENTAL RETARDATION
} Evolving Status

FIG. 13. Hyperornithinemia (Shih et al, 1969); Cardinal initial and evolving clinical pattern.

The patient of Shih was referred at 16 months of age as a mental retardate afflicted by myoclonic seizures. His history, however, suggested manifestations of underlying ammonia intoxication from early infancy. Milk and other protein foods had been resisted. His consistent irritability frequently culminated in attacks of screming, although alternating with phases of lethargy and ataxia (Fig. 13).

The blood ornithine was nine times normal (0.915 $\mu$mole/ml, the normal figure being up to 0.102 $\mu$mole/ml) while that of ammonia exceeded 150 $\mu$g/100 ml. The urinary excretion of homocitrulline was also found to be increased, and this was shown not to be of dietary origin. It is true that sterilization of milk leads to transformation of citrulline to homocitrulline, to be then excreted by infants fed such formula. In this child, however, it did not derive from this particular source, since it continued to be excreted despite feeding with fresh cow's milk.

Not only could this excretion be eliminated by lowering the protein intake to 1.5 gm/Kg/day, the hyperammonemia was likewise reduced, as well as the seizures and the irritability. Apart from so clear a clinical-biochemical co-relationship with protein intake, neither enzymatic nor other basis could be defined.

### *G. Hyperglycinemia, Ketoacidosis, and Hyperammonemia*

A degree of hyperglycinemia and ketoacidosis in early infancy has been regarded as relatively nonspecific within several different metabolic disorders, including the congenital hyperammonemia of CP synthetase deficiency, and vitamin $B_{12}$-unresponsive methylmalonic acidemia.

A close relationship between the metabolism of glycine, ammonia, and short-chain fatty acids, however, is illustrated in a patient recently described by Kirkman. At one month, there was already conspicuous failure to thrive, as well as tremulousness and a history of projectile vomiting, etc., at first suggestive of pyloric stenosis. Significant blood ammonia excess (366 $\mu$g/100 ml) was soon identified, in addition to the acidosis, and the neutropenia so often characterizing hyperammonemic situations. This was lowered to a range of 80–260 $\mu$g/100 ml by reduced protein feeding (1.5 gm/Kg/day). In addition to the mild hyperglycinemia, leucine was also high both in blood and urine, and there was increased methylmalonic acid excretion (MMA), unresponsive to $B_{12}$. An associated mild deficiency of liver CP synthetase activity could be secondary to inhibition by MMA, but nonetheless might be making a primary contribution to the basic upset.

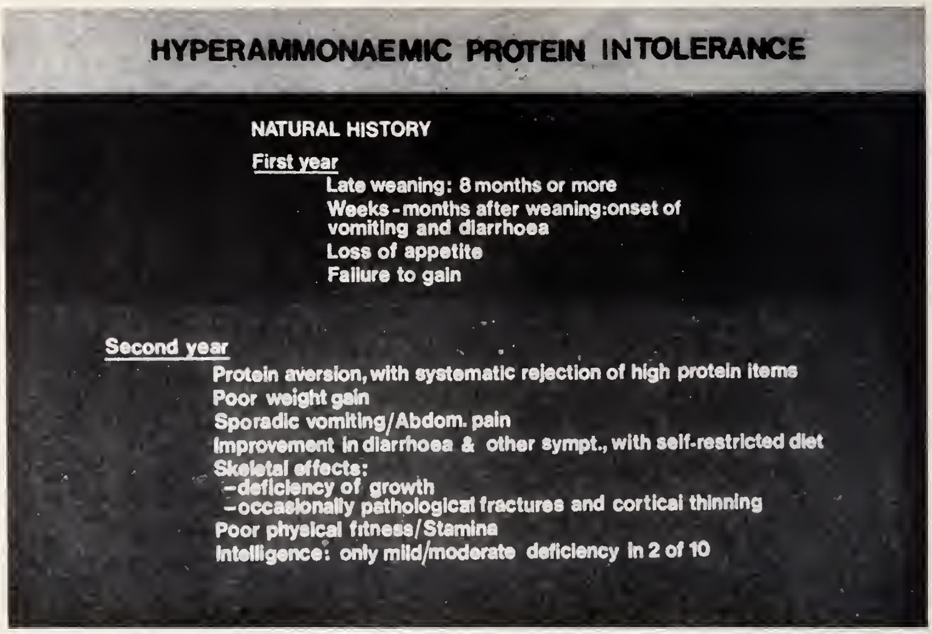
### *H. Protein Intolerance*

An especially interesting clinical syndrome has come into sharp focus since the introduction in 1965 by Perheentup et al of three examples of protein intolerance in infancy associated with a selective defect of transport of dibasic amino acids, mainly arginine and lysine. The infantile intolerance to protein may first be expressed by the onset of vomiting, diarrhea, and hyperirritability within weeks of weaning from the breast to cow's milk with its higher protein content. There is stubborn, all-round anorexia, whereas more specific aversions to particular protein-rich items may emerge in later infancy.

Direct manifestation of the hyperammonemia is probably predominant, as suggested by the lethargy, hypotonia, convulsions, and loss of consciousness (Fig. 14). As in other hyperammonemic states, muscular rigidity may intensify intermittently, sometimes alongside opisthotonic posturing. Stunting of growth is also severe, another consistent feature of childhood hyperammonemia. Liver enlargement always appears at some stage and may be progressive. There may be cellular infiltration of the portal tracts or incipient diffuse cirrhosis. Neutropenia, another frequent concomitant of inborn hyperammonemia, has been found in half of the twelve examples so far accumulated. Again, the variable age of onset is not clearly explicable. In a late-onset type, conspicuous manifestations may also be deferred as much as three to nine years.

An inborn defect of renal tubular reabsorption of lysine and to a lesser extent of arginine has been proposed. Resultant presumptive arginine deficiency has not yet been proven, although it could, paradoxically, be enhanced by ingestion of protein which, in turn, increases the excretion of arginine. Supplementation with arginine can certainly abolish the hyperammonemic responses, while lysine loads augment them.

We have recently been able to prove consistently on repeated automated column chromatography the coexistence of low plasma levels of arginine. We would like to add the findings of no more than trace plasma levels of ornithine,



**HYPERAMMONAEMIC PROTEIN INTOLERANCE**

**NATURAL HISTORY**

**First year**

Late weaning: 8 months or more
Weeks - months after weaning: onset of vomiting and diarrhoea
Loss of appetite
Failure to gain

**Second year**

Protein aversion, with systematic rejection of high protein items
Poor weight gain
Sporadic vomiting/Abdom. pain
Improvement in diarrhoea & other sympt., with self-restricted diet
Skeletal effects:
  –deficiency of growth
  –occasionally pathological fractures and cortical thinning
Poor physical fitness/Stamina
Intelligence: only mild/moderate deficiency in 2 of 10

Fig. 14. Hyperammonemic protein intolerance: Presenting clinical features in relation to age.

as well as subnormal plasma lysine concentrations, while those of citrulline were somewhat higher than normal. This could well be a limiting factor in urea synthesis and hence the accumulation of ammonia.

## *VI. CLINICOPATHOLOGIC ASPECTS COMMON TO ALL THE KNOWN UREA CYCLE DEFECTS*

Unearthing their hyperammonemia has thus helped to expose a surprisingly well-hidden, closely knit community of inborn errors, whether directly of the Krebs urea cycle enzymes or not, which share several important clinical and pathologic aspects in common. Their mutual clinical aspects we have already discussed.

### *A. Relative Hyperammonemic Potential*

Of all the examples cited today, our first homozygous OTC defect appeared to show the most consistently intense concentrations of ammonia both in blood and CSF, even while on a reduced protein intake. It gradually became clear that both categories of congenital hyperammonemia fully lived up to their name in spontaneously sustaining severe ammonia intoxication.

At each enzymatic step beyond these first two, significantly pathologic rises of blood ammonia usually appeared one to three hours postprandially. In ASA, for instance, sharp rises in blood ammonia in the range of 400—500 $\mu$g/100 ml often accompanied exacerbations of vomiting and stupor, etc., following upon infection, high protein feeding, or casein hydrolysate administration. Apart from such events, stress with a loading dose of protein or ammonium salts would only occasionally be warranted in order to establish convincingly the underlying hyperammonemic potential. On the other hand, in the neonatal group of the "early onset" or malignant category of ASA, extreme hyperammonemia may already threaten the infant's life by the fourth day or earlier, even in response to the first small protein feeding.

In presumptive heterozygotes during periods of unmodified protein intake, the fasting levels of blood ammonia were themselves often significantly above upper limits of the normal range, quite apart from the levels induced by protein loading.

### *B. Raised Glutamine Levels in CSF, Blood, and Urine*

In keeping with findings in hepatic coma, the initial CSF levels of glutamine were certainly high in both OTC cases 1 and 2 (2.69 and 1.93 $\mu$moles/ml, respectively). Concurrent plasma levels showed less elevation and could be eliminated by a low protein diet. The same pathognomonic excesses of glutamine in CSF, blood and urine have been reproduced in ASA. Even in the far milder and presumptively heterozygous examples, such as the mother and twin of Case 2, high plasma glutamine may be defined during migrainous accentuations.

These abnormal concentrations of glutamine at all levels studied were taken to reflect maximal activity of the $\alpha$-ketoglutarate-glutamic acid-glutamine system in response to persistently high peripheral and brain levels of ammonia. Glutamic acid metabolism is then almost wholly driven in the direction of glutamine. The underlying ammonia-binding mechanism essentially involves the uptake of two molecules of ammonia by one molecule of $\alpha$-ketoglutarate and one of glutamic acid to form glutamine. This system, although active in muscle and elsewhere, is of the greatest primary importance to the brain, which appears to be dependent upon it for preventing the accumulation of more than trace amounts of free ammonia therein. What proportion of the raised CSF or even of plasma levels of glutamine in our cases stems from its hyperactive cerebral production is yet to be determined. Certainly, glutamine is considerably increased in the brain as well as the cerebrospinal fluid in hepatic coma.

The reactions of this system are themselves directly ATP-dependent. In addition, there is Bessman's postulate that cerebral $\alpha$-ketoglutarate is being drained off to form glutamate and glutamine. Oxaloacetate is also diverted, in the brain, to the manufacture of glutamine. Thus, not only would the compensatory activation tend to slow down function of the citric acid cycle by substrate depletion, but it could impede the rebuilding of ATP. Such secondary ATP depletion could, in turn, handicap ammonia-binding capacity. It was to help combat this effect, and its possible basic deprivation of $\alpha$-ketoglutarate, that we began therapeutic exploration with supplements of citric acid cycle intermediates.

### C. *Abnormal Transaminase Activity*

An initially disconcerting feature in our OTC cases had been the consistent normality from the outset of the customary liver function tests, with the exception of clearly excessive SGPT and SGOT levels. The latter were accentuated not only during crises, but also after increases in protein intake, or when alkalosis was induced (the SGPT rising above 1000 units on one such occasion in our Case 2). The most intense rises both in SGOT and SGPT were observed to accompany crises in the fifth OTC case, reaching 2,200 and 2,600 units, respectively. Other than OTC defects, perhaps the most extreme example of such peak transaminase activity marked the second case of citrullinemia wherein the SGOT reached 1280 units. A clear, positive correlation between increased SGOT and blood ammonia was also seen in ASA with the most significantly high levels characterizing the neonatal forms.

Instead of accepting these levels as principally an expression of hepatotoxicity, the question arises as to whether they instead reflected some compensatory hyperactivity of transamination, a possible alternative mechanism of urea formation or of glutamic acid-ketoglutarate conversion. SGOT is believed to have a role in catalyzing the conversion of L-glutamic acid derived from the ornithine-glutamic semialdehyde shunt into $\alpha$-ketoglutarate. Depletion of the latter by virtue of the excessive need for ammonia coupling or disposal, could be the stimulus for this production. The striking hyperactivity of these enzymes on liver assay which was included in the report on lysine intolerance by Colombo may have an analogous basis.

### D. *Abnormal Excretion of Metabolites of Pyrimidine Synthesis and Catabolism*

Preliminary support was soon to be uncovered in the way of this interesting phenomenon, following initial explorations of alternative pathways of disposal of excessive accumulation of ammonia, having in mind that ammonia is also an important nitrogen substrate in the line of pyrimidine biosynthesis. We were also seeking to unearth hitherto unknown toxic side-effects. Carbamyl phosphate may, in turn, be envisaged as a key substrate common both to the urea cycle and the pyrimidine line of synthesis (Fig. 15). Thus, in a preliminary exploration of the latter, an excess of urinary orotic acid was first identified with ultraviolet exposure of the amino acid paper chromatograms of our second OTC case.

This recalled a much earlier finding of a copious crystalline deposit in the routine urinary examination of a patient during an initial hyperammonemic state. This material had been preserved and now recrystallization several times from water proved it to be orotic acid.

Subsequently, the urinary output of orotic acid appeared to be correlated with the protein intake. While on a low protein intake (that is, up to 0.5 g/Kg) none was excreted by OTC Case 1. On raising the protein intake to 1.0 g/Kg, urinary orotic acid ranged from 18 to 35 mg/100 ml. Total absolute excretion reached about 60 mg per day.

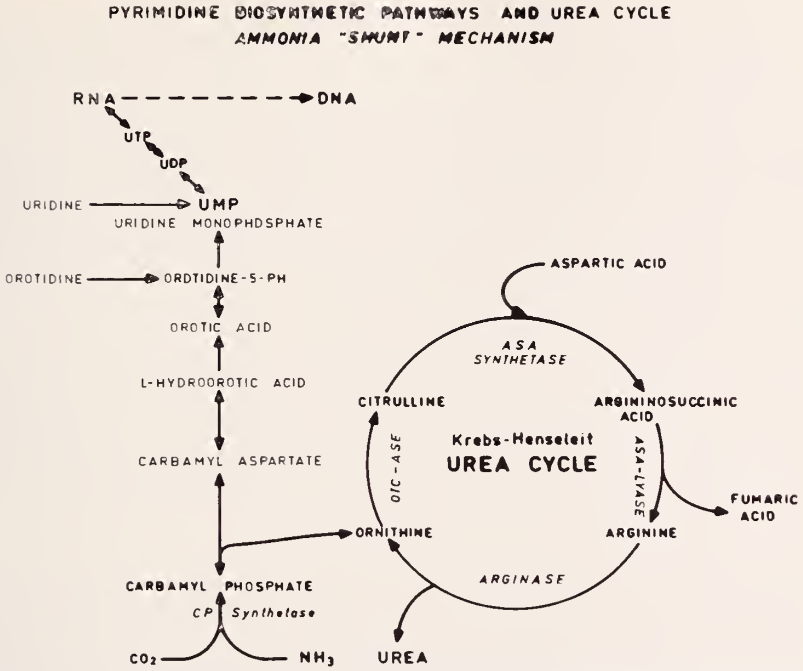

PYRIMIDINE BIOSYNTHETIC PATHWAYS AND UREA CYCLE
AMMONIA "SHUNT" MECHANISM
RNA
DNA
UTP
UDP
URIDINE
UMP
URIDINE MONOPHOSPHATE
OROTIDINE
OROTIDINE-5-PH
OROTIC ACID
L-HYDROOROTIC ACID
CARBAMYL ASPARTATE
CARBAMYL PHOSPHATE
CP Synthetase
$CO_2$
$NH_3$
ASPARTIC ACID
ASA SYNTHETASE
CITRULLINE
ARGININOSUCCINIC ACID
Krebs-Henseleit
UREA CYCLE
OTC-ASE
ASA-LYASE
FUMARIC ACID
ORNITHINE
ARGININE
ARGINASE
UREA

Fig. 15. Involvement of this pathway of pyrimidine biosynthesis, with possible interference in nucleotide metabolism, reflected in the orotic aciduria, uridinuria, and uraciluria found in congenital hyperammonemia and ASA.

During further clarification of the pyrimidine derivates being excreted, excess both of uridine and uracil was also detected. The amounts excreted in OTC Case 1 reached 27 mg/100 ml. This level could be significantly reduced or eliminated by a low protein intake (0.5 gm/Kg/day).

Ammonia loading of the pyrimidine line of synthesis may thus initially be the consequence of its first operating as a "shunt" mechanism hinging upon carbamyl phosphate, and directly diverting ammonia accumulations from the Krebs urea cycle. Ammonia "flooding" may be associated with induction of the initial enzymatic steps of this pathway, and to enhanced and perhaps progressive build-up of its intermediate substrates and abnormal amounts of orotic acid, uridine and uracil in the urine.

The facet of overriding synthesis of pyrimidine nucleotide may in part explain apparently normal developmental progress in the first four to five months of life. But the suspicion remained that the excess of orotic acid and uridine, together with the probable catabolite of the latter, uracil, as found at least in congenital hyperammonemia and ASA, may reflect more than a mere direct sequel of ammonia "flooding." One must bear in mind the profound neurotropic pathology in the homozygotes despite their normal liver histology. Cerebral "atrophy," as a compound of inhibition of cerebral growth and degeneration, is also shared by ASA, in which an abnormal urinary excretion of orotic acid uridine, and uracil has also been defined.

Is there a possibility, on the other hand, that ammonia excess acts through inhibition, enzymatic or otherwise, beyond UMP so that nucleotide uptake into neuronal RNA is disturbed?

It has been postulated that uridine and uracil excess may reflect some specific enzymatic inhibition or distortion beyond uridine in the synthesis of precursors of neuronal RNA. In consequence, a specific type of "pyrimidine starvation" could arise, with the perhaps highly specific involvement of specialized and susceptible groups of neurons. That this may be the basis of a subtle and hitherto unknown pathogenic factor in ammonia toxicity has been under our experimental study for several years.

First, we have confirmed that orotic aciduria is a regularly reproducible consequence of acute ammonia intoxication in the rat. Second, the parallel and abnormal orotic acid accumulation in the liver has been isolated and purified. Finally, a variety of experimental evidence has been elicited, using radioisotopically tagged orotic acid, to indicate that acute hyperammonemia does lead to 60 percent depression of orotic acid uptake into RNA.

### *E. Characteristic Electroencephalographic Effects*

This field of study has produced yet another relatively specific parameter of the neuropathic toxicity of hyperammonemia. A diffuse EEG dysrhythmia has now been noted in all these defects. There is a predominant slowing of the basic rhythm from the normal alpha rhythm, with moderate to high voltage, bilaterally synchronous, and symmetrical bursts of slow delta waves, occurring at the rate of two to five per second. This pattern has been regarded as characteristic of early stages of impending hepatic coma. Initially they appear in the frontal region. Their predominant background and paroxysmal slow activity were not only constant in some of these, they could also be either induced or clearly accentuated during protein and ammonia loading as well as during the climax of episodic clinical attacks. Conversely, the reversibility of these changes during or after a period of effective low protein therapy has been reassuring.

# Infection and Immunity in Alcoholic Cirrhosis

BERNARD STRAUS, M.D.* AND MAGDALENA R. BERENYI, M.D.†

Increased susceptibility to infection constitutes a constant threat to the patient with alcoholic cirrhosis. The biochemical and pathologic disarray found in the cirrhotic state involves not only the liver but the blood cells, the bone marrow, humoral factors including immunoglobulins and complement, cellular immunity, vitamins, and the many complexities associated with altered protein synthesis. In varying measure, disturbances in these components of a viable body economy constitute the phenomenon of resistance and render the cirrhotic patient a prey to a number of infections. In one large series, these included pneumonia, peritonitis, septicemia, erysipelas, tuberculosis, and pyelonephritis (1). Others have reported similar findings (2–5). The cirrhotic is also predisposed to gram negative sepsis (6).

The cause for this increased incidence of infection is not readily apparent. There are probably multiple factors. It seems quite clear that the basis for diminished resistance is not due to impaired antibody production. Serum globulins are increased in alcoholic cirrhosis. The rise includes the immunoglobulins, IgM, IgA and IgG (7). IgA elevation has been reported to be relatively more striking in alcoholic liver disease (8). These immunoglobulins do not appear to be qualitatively abnormal. Havens showed an enhanced capacity of patients with chronic liver disease to produce antibodies to bacterial antigens such as diphtheria and tetanus toxoid. Patients with chronic hepatitis and cirrhosis produced two to three times as much antitoxin as did controls and the highest levels were in patients with more advanced cirrhosis (9).

### *Immunologic Enhancement*

It is superficially paradoxical that cirrhotic patients who have an increase in humoral antibody should have diminished resistance to infection. This is readily explained since there are multiple factors in immunity. In addition, the interesting phenomenon of immunologic enhancement may be responsible in part. An important property of humoral antibodies is their ability to interfere with cellular immunity directed against tissue homografts or antigenic extracts. Gamma globulin may be involved by combining with isoantigenic determinants on the target cell surfaces of the organisms, protecting them from interaction with host immunologically competent cells. Parish has shown a significant relationship between cell-mediated immunity, humoral antibody formation, and immunologic tolerance. By acetoacetylating Salmonella adelaide flagellin, a

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series of flagellin preparations were obtained with reduced antigenic properties. This loss in antigenic activity was accompanied by a reduced capacity to initiate primary antibody formation and impairment of immunologic memory but an enhanced ability to produce cell-mediated immunity and immunologic tolerance (suppression of antibody formation) (10). It was concluded that antibody formation and cell-mediated immunity in certain systems are competing immunologic processes. A strong cell-mediated immunity is accompanied by antibody suppression ("tolerance"), and conversely Parish believed that a strong antibody response would produce a suppression of cell-mediated immunity. Antigenic activity is the factor that determines which response predominates. Although antibody formation and cell-mediated immunity may be opposing immunologic processes, it is clear that both immune mechanisms may be suppressed in the same individual. Thymomas may be associated with immunologic deficiency both of cellular and humoral systems (11, 12). Gatti et al have described a syndrome of short-limbed dwarfism in patients who had very low levels of immunoglobulin and severe deficits of cell-mediated immunity. This combination has proven incompatible with life (13). Another effect may be a central action on the host's cellular immunologic response machinery impairing the ability of its cells to develop cellular immunity (14). Interfering with this mechanism of "immunologic enhancement" presents therapeutic possibilities. A serum which could control the humoral immune response would prevent inhibition of the cellular immune response, thereby preventing certain infections. Such a serum is presently being developed. It is an "antiplasmacyte serum" (APS). By preventing the plasma cells from producing antibodies, APS would act in a similar fashion to anti-lymphocyte serum which has been effective in impairing the delayed hypersensitivity reaction and in preventing the rejection of transplants.

Another explanation has been suggested by Papageorgiou and Glade (15). Since cell-mediated immunity is the most important means by which the host resists intracellular parasitism by certain microbial and viral agents, impairment of such immunity would decrease the capacity of the organism to remove cells containing intracellular microorganisms. This would permit increased replication of these agents and their antigens, resulting in an increased antigenic challenge presented to the antibody-forming organs of the host. The result would be an increase in humoral antibody formation.

### *Other Factors in Resistance*

Anatomic barriers are important in resistance to infection. These include skin, mucous membranes, and other structures which limit penetration and dissemination of microorganisms. Injury to these tissues due to trauma or inflammation destroys these barriers. An obvious example is the super-imposition of bacterial endocarditis on damaged cardiac valves. Portal-systemic anastomoses in cirrhosis enable the blood to bypass the liver and prevent the trapping of antigen.

Phagocytes such as the polymorphonuclear leukocytes and macrophages are able to engulf bacteria efficiently only if serum factors, including opsonins and/or antibodies, are present. Thus, effective host resistance requires both humoral and cellular factors. Patients with agammaglobulinemia or with insufficient phagocytes, as in agranulocytosis or with leukopenia from a variety of causes, have a greatly increased susceptibility to infection. It is noteworthy that recently alcohol has been found to inhibit phagocyte mobilization (16). In cirrhotic rats Escherichia coli has been shown to persist and proliferate in the tissues of the liver, lung and spleen indicating impairment of bactericidal activity in these tissues (17). Recently DeMeo and Anderson found that polymorphonuclear leukocyte chemotaxis was markedly impaired in patients with alcoholic cirrhosis of the liver (2). The defect was associated both with a serum inhibitor of chemotaxis and a deficit of known complement components. Intraleukocytic killing of phagocytized C. albicans was not significantly impaired.

The complement system in serum has the capacity to produce irreversible damage to cell membranes. It is activated by interaction with antigen-antibody complexes or gamma globulin aggregates. Complement combines with gamma globulin to precipitate soluble antigen-antibody complexes which react with cell membranes. Complement is not merely hemolytic. A large variety of cells of mammalian origin and certain microorganisms are susceptible to its toxic action. Complement plus antibody act to kill gram negative organisms. A qualitative defect in C5 has been found to be implicated in recurrent gram negative infections. Such infections are common in patients with advanced Laennec's cirrhosis who often have depressed serum complement (18).

Properdin was described by Pillemer and his colleagues in 1954 as a newly discovered serum factor that represented an important mediator of "nonspecific immunity" (19). It was subsequently shown that at least some of the activities of properdin were antibody- and complement-dependent and doubt was thrown both on the significance of properdin and even on its existence. The purification and characterization of properdin as a protein put an end to the controversy as to its existence. It is a nonimmunoglobulin serum beta-globulin with a sedimentation constant of 5.2S and a molecular weight of around 230,000. In conjunction with various naturally occurring antibodies, components of complement and Mg, it comprises "the properdin system" which is believed to play a part in the killing of certain bacteria, the neutralization of some viruses, and the hemolysis of erythrocytes of patients with paroxysmal nocturnal hemoglobinuria. The mechanisms of action involved and the significance of properdin as a factor in immunity remain to be clarified. Properdin may act by activating the alternate pathway of complement (20, 21).

Muramidase (lysozyme) is an enzyme which is capable of lysing certain bacteria by attacking cell wall peptides. It occurs in body fluids including the serum and plasma of man and animals. The richest source is egg-white. Activity is determined by the lysis of a gram positive coccus. The enzyme kills gram positive organisms. It enhances the bactericidal effect of antibody and complement. Beta lysin is another serum factor which is also known as the "beta bactericidal system." This substance is capable of killing a number of different gram positive organisms. The role of these factors in cirrhosis of the liver has not been established.

Infection with some viruses stimulates the local production of a protein or group of proteins known as interferon. Noninfected cells exposed to interferon are protected against viral infection. This substance may be the mechanism for the termination of infection and the repair of inflamed tissues before specific antibody is formed. Stimulation by migration inhibition factor may be the mechanism for the release of interferon and for the nonspecific cellular immunity shown by specifically activated macrophages.

### *Role of Nutrition*

Popper and Schaffner stated that increased susceptibility to infection in alcoholic cirrhosis is a reflection of protein deficiency (22). It is known that even relatively short periods of starvation render experimental animals unusually susceptible to bacterial infection. Imbalance of diet, especially shortage of protein, acts directly through hormonal changes or alterations in intestinal flora. Apparently, malnutrition does not affect antibody formation, phagocytosis, or the inflammatory reaction (17). While Sherlock noted that protein loss into the gastrointestinal tract in cirrhotics is not greater than in normal subjects, the loss in grams per day represents a larger than normal fraction of the total albumin synthesized. This loss correlates with the severity of liver disease (7).

Protein-calorie malnutrition depresses cell-mediated immunity. Woodruff demonstrated such depression in malnourished children (23). Marasmic animals have an extreme atrophy of thymus, lymph nodes, and spleen, and have severe lymphocytopenia. In a study by Chabner et al, the role of malnutrition in decreasing cellular immunity was emphasized (24). Low levels of pyridoxal phosphate (PALP) have been suggested as the cause for anergy in patients with Hodgkin's disease. PALP is derived from pyridoxine. A deficiency of this vitamin in association with malnutrition in patients with alcoholic cirrhosis could account for depressed cellular immunity but has not yet been studied. Tryptophan metabolism was found to be altered in chronic alcoholics without decompensated liver disease. Depression of conversion of tryptophan to 5-hydroxyindoleacetic acid (5-HIAA), the urinary metabolite of serotonin, was observed. Decreased 5-HIAA levels persisted despite improvement in nutrition or abstinence from alcohol (25). In a recent study we found that absence of delayed hypersensitivity reactions (DHR) could be correlated with the serum protein levels in most instances, and particularly with depression of serum albumin (26). This has been confirmed in a more extended study (27). Portal or alcoholic cirrhosis was long considered a nutritional cirrhosis in which inadequate diet played an important part. The studies of Rubin and Lieber have emphasized the toxic role of alcohol in pathogenesis and minimized the role of malnutrition (28). Protein undernutrition as exemplified by kwashiorkor is not satisfactory evidence for the role of protein-calorie undernutrition in the pathogenesis of cirrhosis according to Popper and Schaffner, since kwashiorkor produces a fatty liver in children with some portal fibrosis, but not with cirrhosis (29). However, there is evidence that the impaired nutrition of the cirrhotic may be an important factor in susceptibility to infection. Scrimshaw found that improved nutrition reduced the frequency and severity of infectious disease (30). Conversely, it is well known that acute nutritional diseases such as kwashiorkor, beriberi, scurvy, the keratomalacia of avitaminosis A, and nutritional anemia seem to be precipitated by a prior episode of infectious disease in persons of borderline nutritional status (31). Thus there is a synergism of malnutrition and infection.

### *Cellular Immunity*

Present evidence indicates that delayed hypersensitivity reactions (DHR) or cellular immunity is important for defense against most viral, fungal, protozoal and many bacterial diseases including tuberculosis. However, Uhr disagreed and has stated that there is insufficient evidence to support the view that delayed hypersensitivity is a factor in immunity to certain infectious agents (32). Among the convincing pieces of evidence which support the role of cellular immunity in certain infections is the disease known as congenital thymic aplasia or DiGeorge's syndrome. Infants with this syndrome exhibit increased susceptibility to viral, fungal, and bacterial infections which may be overwhelming. Serum immunoglobulins are normal but there is absence of DHR to the usual skin antigens, and skin allograft rejection is absent or abnormally delayed. In contrast to DHR, humoral antibody plays a primary role in immunity against acute bacterial infections such as those due to streptococci, pneumococci, staphylococci, and such viral diseases as infectious hepatitis.

Since patients with Laennec's cirrhosis have increased susceptibility to fungal infections such as candidiasis, and a variety of other infections including tuberculosis, it seemed reasonable to determine whether this was associated with or was a result of impaired delayed hypersensitivity. In a study of fifty patients with alcoholic cirrhosis of the liver, 32 percent of the patients could not be sensitized to dinitrochlorobenzene (DNCB) (26). Since it is generally accepted that DNCB is probably the most powerful stimulus to the delayed hypersensitivity reaction, a 32 percent negative result in this group of patients is clearly of importance. This is approximately six times the expected negative result of five percent. The diminished number of patients who could be sensitized to DNCB as well as the reduced number of patients with DHR to candida and streptokinase-streptodornase suggests impairment in the cellular response in alcoholic cirrhotic patients. Tobias, Safran, and Schaffner studied lymphocyte transformation in 14 patients with various liver diseases using the patients' own liver tissue obtained by liver biopsy as antigen (33). Lymphocyte transformation is a reflection of cellular immunity. Two patients of four with primary biliary cirrhosis and one of two with chronic active hepatitis developed positive lymphocyte transformation reactions. The other patients, including three with inactive alcoholic cirrhosis, failed to show transformation. This demonstrated that the patients who failed to react, including the alcoholic cirrhosis patients, did not have autoimmune reactions. In vitro tests to determine DHR to foreign antigens were not done.

### *Specific Disorders Associated with Cirrhosis*

*Tuberculosis:* This disease has been frequently cited as a common complication of cirrhosis. Rolleston and McNee reported in 1929 that in 12–14 percent of patients with cirrhosis, death was due to pulmonary tuberculosis (3). Others have reported similar findings. Tuberculous peritonitis is especially common. In a series of 47 cases of tuberculous peritonitis, Burack and Hollister reported 20 of the patients had cirrhosis of the liver with ascites (4). Increased susceptibility to tuberculosis is reflected in the increased number of patients who have a positive PPD reaction. In one series 65 percent of patients with alcoholic cirrhosis of the liver were positive. This is significantly higher than the general population. Why does not the impairment of the DHR in alcoholic cirrhosis suppress the reaction to PPD? Several reasons may be advanced. An established immune response is considerably more resistant to suppression than a developing response. Most agents lack significant activity against either the differentiated antibody-producing cells or cells carrying immunologic memory (34). A higher incidence of reinfection in this population may overwhelm any suppression of the PPD reaction due to cirrhosis of the liver. Since patients with DHR reactions such as a positive PPD are obviously susceptible to reinfection, factors other than impaired cellular immunity must be responsible. Antibodies, according to Raffel, are probably not involved (35). Lysozymes have been suggested as a factor. Macrophages which engulf mycobacteria have an inhibitory effect. Immunity to tuberculosis may be a nonspecific activation of cells of the reticuloendothelial system. Unusual macrophages with extraordinary tuberculostatic properties have been found to accumulate in masses in the lungs of vaccinated animals following intravenous injections of attenuated tubercule bacilli. These cells are not lymphocytes, but there may be developmental connections between these two cell types. The implication is that acquired immunity in tuberculosis may not be part of the DHR but rather a concomitant event (35). Another suggestion is that immunity may depend on delayed reactivity to a lipopolysaccharide antigen of the organism rather than to its protein antigens (36).

*Salmonellosis and Other Gram Negative Organisms:* The role of the liver in filtering intestinal organisms from the blood is well known. Eighty percent of the portal blood passes through the liver (37). Portal-systemic shunting in chronic liver disease is largely responsible for decreased extractions of bacteria by the liver by permitting the circulating bacteria to bypass the hepatic reticuloendothelial system. Animal studies have confirmed the hypothesis that clearance of blood-borne bacteria by the hepatic R-E system is diminished in cirrhosis of the liver. It is not surprising, therefore, that salmonellosis has been encountered with increased frequency in patients with cirrhosis of the liver. A frequent association between salmonellosis and jaundice has also been reported (38). It has been pointed out by Bennett and Hook that salmonellosis is more apt to occur with poor sanitary conditions that favor the spread of viral hepatitis (39). The increased incidence of salmonella infection complicating chronic liver disease could be related to the increased transmural migration of bacteria. Certain metabolic factors, alkalosis, potassium deficiency, and arterial $O_2$ desaturation may also contribute to the increased incidence. With the use of a vasopressor substance, prolonged arterial constriction has been implicated.

*Hyperglobulinemia:* The pathogenesis of the hyperglobulinemia regularly seen in patients with cirrhosis of the liver is uncertain. The normal liver synthesizes little or no gamma globulin. Gamma globulin is derived chiefly from the plasma cells and possibly from some lymphocytes and is synthesized in the spleen, bone marrow, lymph nodes, and Peyer's patches of the intestine. The normal liver lacks plasma cells and hence makes little or no antibody. However, Paronetto, Rubin, and Popper have identified gamma globulin in the hepatic mesenchymal cells in cirrhosis (40). Protell found high titers of antibody globulin to salmonella in patients with chronic active hepatitis (41) and Bjørneboe found higher titers of E. coli antibodies in patients with cirrhosis of the liver than in controls (42). Furthermore, patients with portacaval shunts had high titers and their immunoglobulin levels were generally higher. The normal liver may protect the body against sensitization to dietary and bacterial antigens absorbed into the portal system. When Kupffer cells are damaged or bypassed by shunting, some antigens absorbed from the gut may become available to stimulate excessive antibody formation which is reflected by an elevated immunoglobulin level. Studies by Glagow, Kent and Popper suggest that cells with cytoplasmic pyroninophilia of spleen and abdominal lymph node origin contribute to the production of the hypergammaglobulinemia of the cirrhotics (43).

*Peritonitis:* Factors that render the cirrhotic patient prone to develop peritonitis are the failure of hepatic removal of bacteria and vascular abnormalities. Portal-systemic shunts are responsible for decreased extraction of particulate matter by the liver. Here, too, it has been shown by Beeson et al that shunting permits the circulating bacteria to bypass the hepatic reticuloendothelial system and the spleen, both of which are major sites for removal of bacteria from the blood (44). Studies in animals have confirmed the hypothesis that clearance of blood-borne bacteria by the hepatic reticuloendothelial system may be diminished in cirrhosis (45). Decreased hepatic removal tends to perpetuate bacteremia. Primary bacteremia due to coliform organisms is a common complication in cirrhosis. Subacute bacterial endocarditis seems to be most common. Spontaneous peritonitis and bacteremia in patients with alcoholic cirrhosis of the liver caused by enteric organisms have been described by Conn (46). Careful analysis of the hospital course of patients with this disorder did not reveal any single precipitating cause common to all. This syndrome is characterized by the abrupt onset of fever, chills, and upper abdominal pain. The symptoms are followed by hypotension and coma. It was postulated that decreased resistance of the intestinal mucosa permits the escape of bacteria that infect the ascitic fluid.

*Candidiasis:* Systemic candidiasis has been described in association with cirrhosis of the liver. Infections with C. albicans, the most common of the candida species, develop in many different settings. Candidiasis ranges from a mild self-limited superficial infection to an overwhelming systemic disease with poor prognosis. Alteration in host defense is common in many of the different clinical situations associated with this infection. There are many factors that favor candida invasions. Skin scarring, superficial wounds, imbalance in host and parasite relationship, and the use of broad spectrum antibiotics are among the more prominent ones. The development of candidiasis is particularly apparent in clinical conditions resulting in severe malnutrition (47). Among these, of course, is alcoholic cirrhosis of the liver.

## Summary

Abundant evidence exists that patients with alcoholic cirrhosis of the liver have increased susceptibility to infections. These infections may be caused by gram positive or gram negative organisms, tubercle bacilli, or fungi. Undoubtedly, multiple factors are involved in this increased susceptibility. Among them are malnutrition, impairment of protein synthesis, immunologic enhancement, shunting, intestinal permeability, impaired phagocytosis, deficient leukocytic chemotaxis, and depressed serum complement. Evidence has been cited implicating impairment of cellular immunity as a factor in increased susceptibility to infection. In part, this may be related to protein-calorie malnutrition. Defective protein synthesis may also be important since some correlation between hypoalbuminemia and impaired cellular immunity has been shown. On the other hand, no evidence exists to indicate that alterations in the immune response play any part in the pathogenesis of alcoholic cirrhosis of the liver.

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# Therapy of Herpes Simplex Virus Infections: A Prospective View

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Herpes simplex virus infection is ubiquitous in man. The virus is the etiologic agent of cold sores and fever blisters of the skin, a painful keratitis of the cornea of the eye, a common venereal infection in men and women, and is a frequent cause of severe, often fatal encephalitis (1–5). The source of virus for these acute infections is often the afflicted individual himself; he may harbor a reservoir of latent virus in some place and form for his lifetime (6). In addition, epidemiologic (7) and immunologic (8) studies have shown venereal herpes simplex to be associated with cervical cancer in women. This infection may also transmit a generalized herpetic infection to the fetus or newborn either transplacentally or at birth, often with disastrous consequences (9, 10). In light of the widespread pathology of herpes infection, it is surprising that no effective therapy for it has yet been found.

The purpose of this short review is to present the general biology of herpes simplex virus replication, which usually results in destruction of the infected cell. Emphasis is placed upon areas where immunochemotherapeutic attempts at arresting the cell damage might prove fruitful.

### *Replication of herpes simplex virus in permissive cells*

Herpes simplex virus infects susceptible cells with the production of thousands of progeny virus particles. The kinetics of cellular production of virus are summarized in Figure 1: A known number of cells is experimentally infected with a known number of virus particles. Such cells permit many virus gene functions to be expressed and complete, infectious virus progeny particles are made. These cells are called permissive for virus replication, but not all cells are permissive, and in these nonpermissive cells the virus cycle may be completely (11) or partially (12, 13) aborted after infection.

Once the infecting particle is inside the permissive cell, it redirects cell metabolism so that cell DNA, RNA, and protein syntheses are almost totally shut off within 3 to 5 hours (14–16). Utilizing the machinery of the permissive cell (including membranes, enzymes, ribosomes, precursor pools) the virus then redirects syntheses of these macromolecules so that all produced are virus specific. As all synthetic activity in the cell tapers off and its integrity is finally destroyed, these macromolecules are assembled into thousands of infectious virus particles (17).

One of the first morphologic changes in herpes simplex virus-infected permissive cells is the appearance of a dense band around the internal periphery of the cell nucleus. Figure 2 shows that this band contains the remnants of the cell

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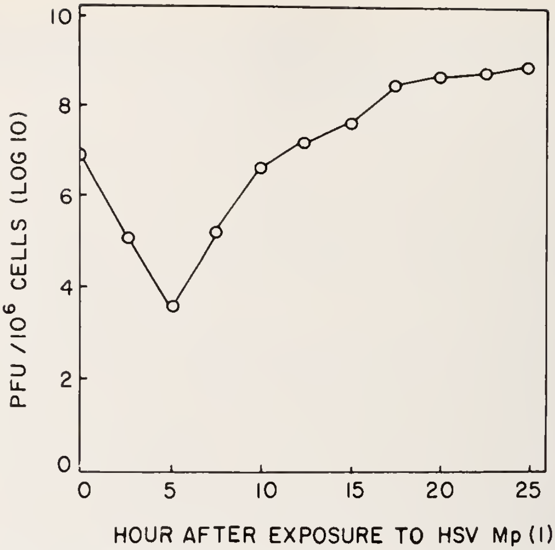

FIG. 1. Production of herpes simplex virus (strain Mp; type 1) by HEp-2 (human epidermal carcinoma) cells in continuous cell culture. Cells were infected for 1 hour at a multiplicity of 10 plaque-forming units per cell.

chromosomes that contain DNA made prior to virus infection. Figure 2 is an electron micrograph prepared using autoradiographic techniques (18–20). Human epidermal carcinoma (HEp-2) cells were labeled with 3H-thymidine (5 uc/ml) for 15 minutes prior to infection with herpes simplex virus. Ten hours after infection cells were fixed and sectioned and photographic emulsion was placed over the sections. After exposure and development, photographic grains (Fig. 2) are localized over the band at the nucleus periphery showing cell DNA made prior to infection is transported to this area. Figure 3 was prepared by similar techniques, however, 3H-thymidine was placed on the cells at 5 hours after infection, a time when all DNA being made is viral (17). Figure 3 shows that viral DNA occupies a lightly staining central area of the nucleus. Exposed photographic grains (arrows) are over this area. Details of cell and virus growth, infection, and assay techniques have been described previously (11, 12, 21).

Within 2 to 3 hours after infection, the first newly synthesized virus-specific antigens are detectable in the cell cytoplasm (22). These antigens are then transported to the nucleus (23) where virus precursor particles are assembled. These particles (Fig. 4A) are 80 nm in diameter, consist of a protein shell probably enclosing DNA, have icosahedral symmetry, and are called virus nucleocapsids. These nucleocapsids are probably not infectious (24, 25), but become infectious after they leave the nucleus by budding through, and being enveloped by, the internal nuclear membrane (Fig. 4B). The enveloped nucleocapsid is then released into the perinuclear space (Figure 4B) and into channels of the endoplasmic reticulum (26) and are transported outside the cell, free to infect neighboring cells.

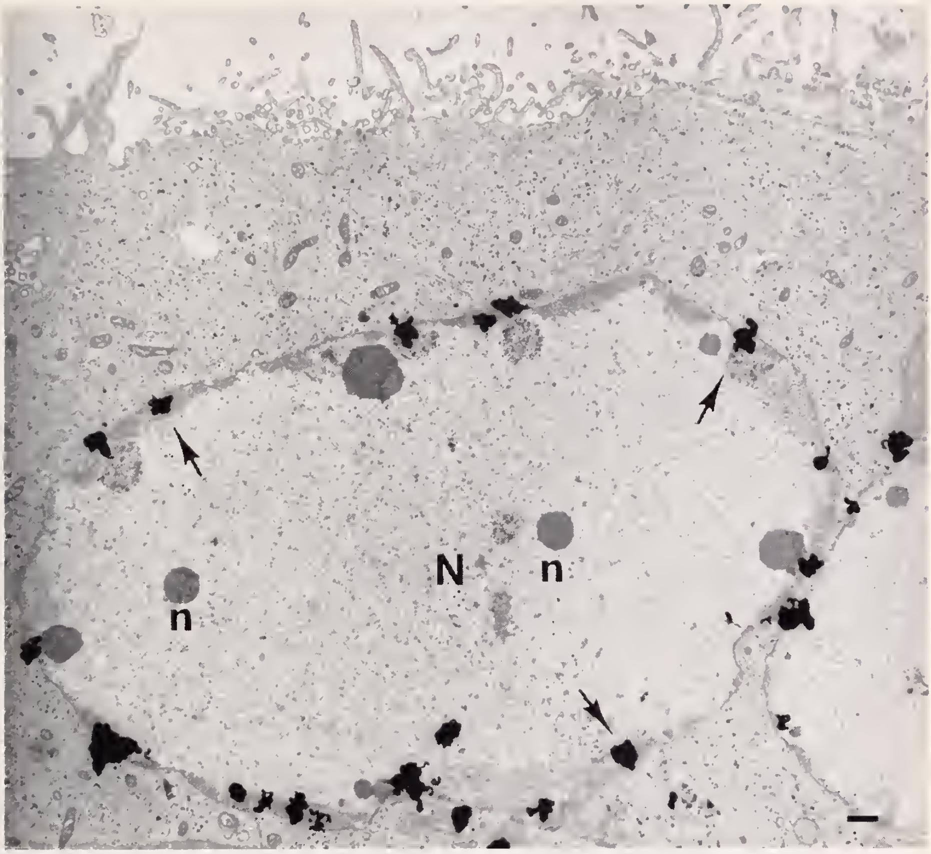

FIG. 2. Electron micrograph of thin section of a HEp-2 cell infected for 10 hours with herpes simplex virus. Before infection, cell DNA was labeled with 3H-thymidine for 15 minutes. By autoradiographic techniques (18–20) it can be seen that cell DNA (arrows) is localized around the periphery of the nucleus (N). Several nucleoli (n) are present in the cell. Bar = 1 micron.

In addition to utilizing release of virus to spread the infection, the virus may spread by inducing cell to cell fusion. Figure 5 shows a syncytia induced by herpes simplex virus infection. The fusion of neighboring cells relies upon the ability of the virus to extensively alter the surface of the cells it infects (27, 28). Figure 6 is an electron micrograph of a HEp-2 cell infected with herpes simplex virus for 10 hours. Before preparation for electron microscopy, uninfected and infected cells were treated with anti-herpes simplex virus serum made in rabbits. The virus used to make the immune serum was grown in a heterologous, noncross-reacting cell line (hamster astrocytes). The cells were then treated with anti-rabbit IgG made in goats commercially coupled to ferritin (Cappel Laboratories, Downington, Pa.). Figure 6 shows that the surface of the infected cell contains virus-specific antigens. Uninfected and other control cells did not react with either serum. The relationship of this change in immunologic specificity of the cell surface to fusion is not firmly established.

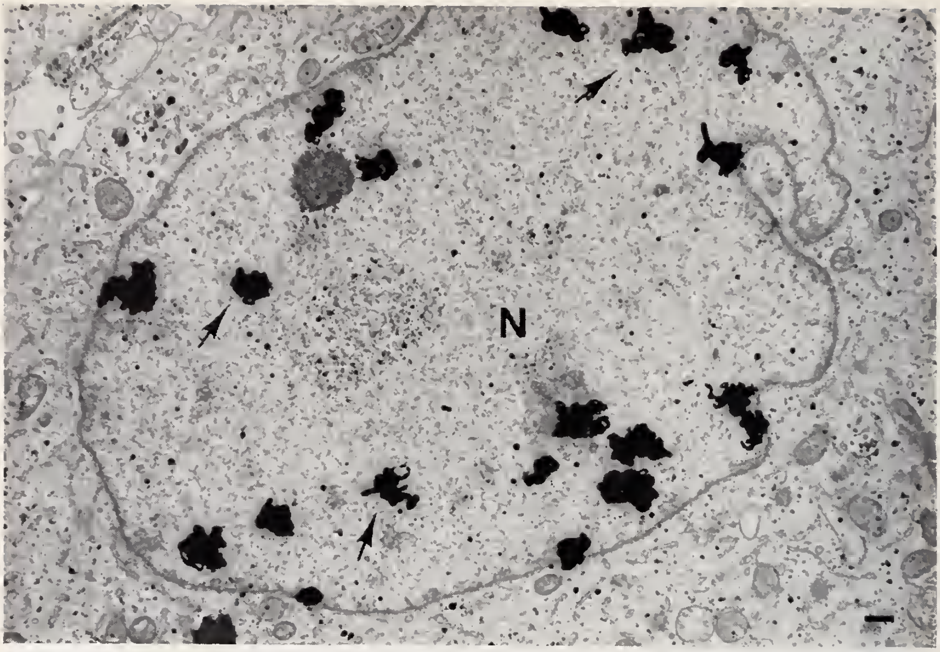

FIG. 3. Autoradiograph of herpes simplex virus infected HEp-2 cell 5 hours after infection. Just prior to fixation, DNA was labeled for 15 minutes with 3H-thymidine. Viral DNA (photographic grains, arrows) is scattered throughout the nucleus (N). Bar = 1 micron.

Transmission of virus from cell to cell by fusion is extremely valuable to the survival of the infection: it permits the virus to spread without being directly exposed to the host's immune defenses against it. Hence the infection may thrive even in the presence of high levels of circulating antibody against it (29).

The virus utilizes the natural resources of the cell very efficiently to produce thousands of copies of itself, toward what would seem to be eventual destruction of the entire organism. Only a delicate balance between the acute phase of infection and the host's immune response to it keeps it in check, however, the infection is probably never completely eliminated, and the individual may be afflicted with recurrent acute episodes of varying severity and consequences for his lifetime.

### *Therapy of acute phases of infection*

The approaches to therapy of the acute, clinical phases of herpes simplex virus infection are summarized in Table I. The data concerning attempts at therapy of the latent phase of chronic infection are very meager, but the same areas of attack have been utilized. As in therapy of all infections, the ideal agent is one which has maximum activity against the infectious material with minimal trauma to the host and normal cells. A therapeutic agent with such selective toxicity has not yet been found.

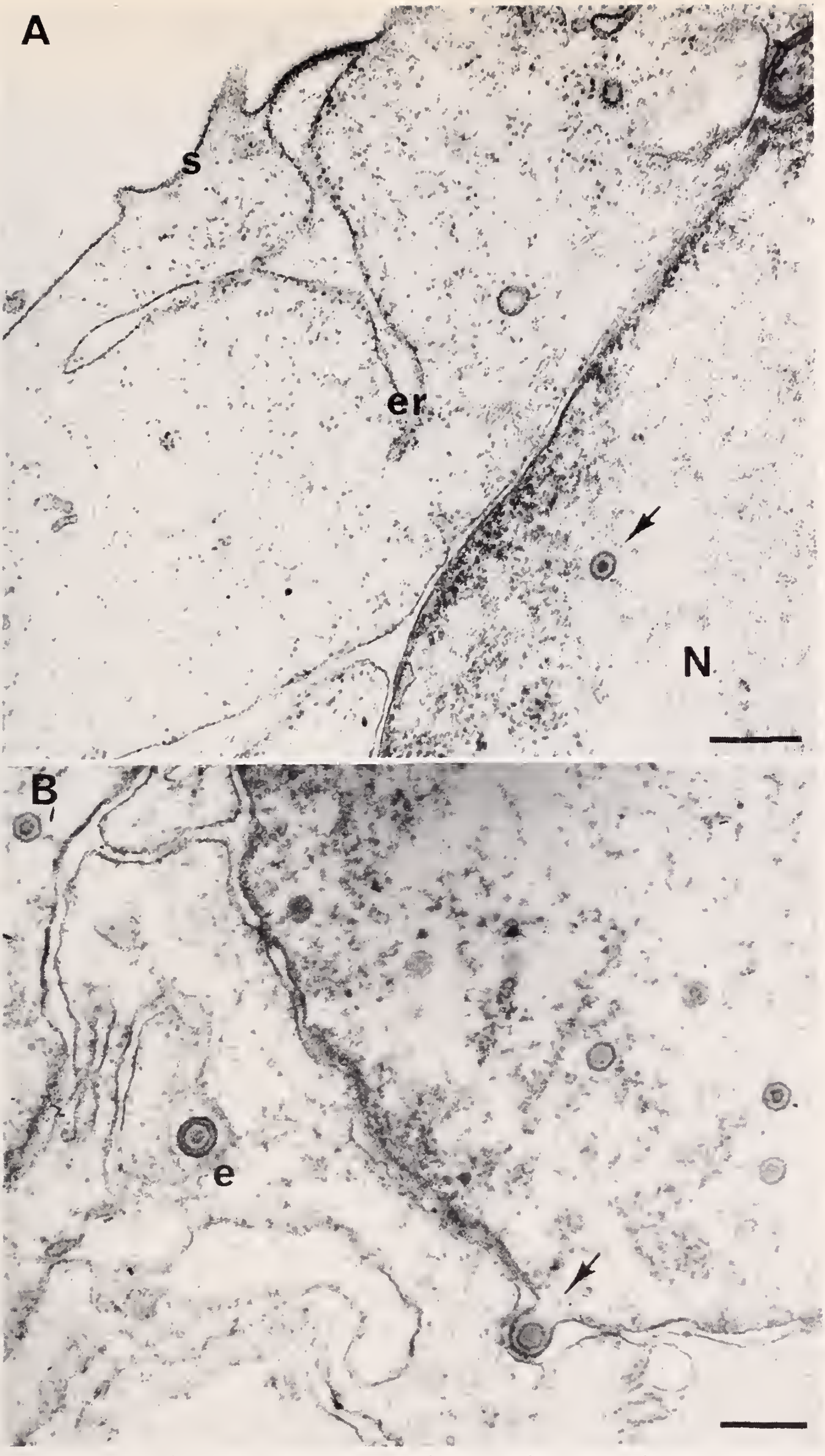

FIG. 4. Electron micrographs of HEp-2 cells infected with herpes simplex virus. A. Viral precursor particles (arrow) are assembled in the nucleus (N). The cytoplasmic channels of the endoplasmic reticulum (er) end in double membrane structures which alter the surface of infected cell (s). Bar = 400 nm. B. Envelopment of viral precursor particle (arrow) by nuclear membrane. Enveloped particles then appear in the perinuclear space and are transported to the outside of the cell. (e) = completely enveloped particle. Bar = 400 nm.

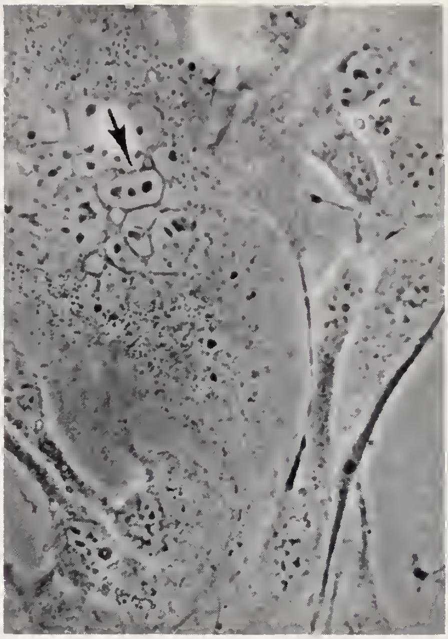

FIG. 5. Giant cell (polykaryocyte) formed by cell fusion after herpes simplex virus infection. Arrow points to nuclei in compact area in the center of the syncytia.

The virus replication cycle presents several aspects which have been exploited for immunochemotherapeutic purposes. By four hours after infection, the DNA being synthesized within the infected cells is exclusively viral. Chemotherapeutic inhibition of DNA synthesis at this time to arrest the production and spread of virus is currently the most successful way to deal with the infection (30). Drugs such as IUdR (5-iodo-2′-deoxyuridine) (31) and ara-C (cytosine arabinoside) (32) nonselectively block DNA synthesis in cells that are actively making DNA. Although these drugs have anti-viral activity, their nonselectivity limits their use clinically when administered systemically as in cases of viral encephalitis; frequently the level of toxic damage from the drug approaches that of the virus (33, 34). Also, mutants of the virus arise which circumvent the biochemical block imposed by the drug (35). In these cases the virus infection may proceed as if no drug were present at all. When topically administered to treat the dermatitis or corneal symptoms associated with acute infection, the drugs are somewhat more effective (36), however, their value is still limited.

Because of the specificity of the antigen-antibody reaction, induction of a passive immunity by administration of immune gamma globulin has been attempted (37). Due to the complex interaction between the infection and the immune system, however, this did not prove to be effective. The administration of steroids (prednisone, methadexasone) both as immunosuppressives and as agents which alter the hormonal balance of the individual does not appear to hasten the onset of remission of acute-phase infections (38).

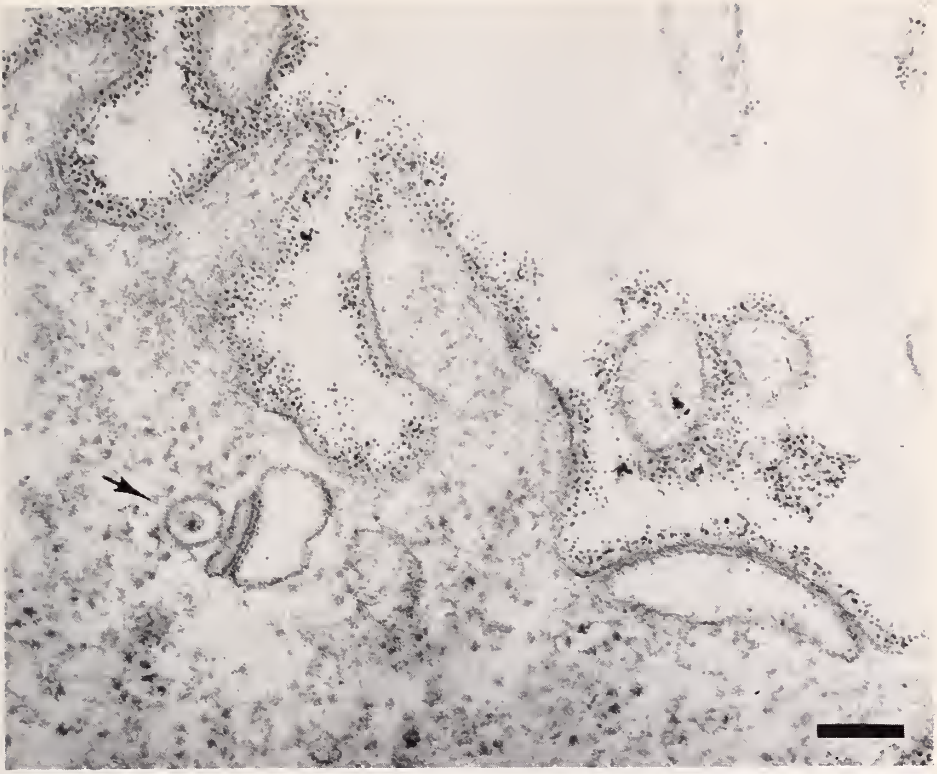

Fig. 6. Electron micrograph of HEp-2 cell infected for 10 hours with herpes simplex virus. Before fixation cells were reacted with anti-herpes serum produced in rabbits from an infected heterologous cell line. After thorough washing, anti-rabbit IgG coupled to ferritin was placed on the cells. It can be seen that after infection, the cell surface strongly reacts with the immune serum. Arrow points to viral nucleocapsid particle in cytoplasm. Bar = 200 nm.

Recently, the administration of poly(I)·poly(c) complexed to (poly-D-lysine) has been shown to be a potent interferon inducer in experimentally infected animals (39). Interferon is an active, very cell-specific viral inhibitor which initially showed little promise of being active against herpes simplex virus infection (40). Newer approaches, however, have proven interferon to be very effective in limiting the extent of herpes infection experimentally and clinically, especially against herpes simplex encephalitis (41, 42). Interferon and interferon inducers may prove to be of use in the management of several acute manifestations of herpes simplex virus infection.

Further refinement of these chemo- and immunotherapeutic techniques, alone or in combination, may show them to be of greater value than they are now.

### *Areas of possible future therapy*

Examination of the way in which herpes simplex virus redirects the cell to make more virus particles while destroying the cell suggests several areas for possible arrest or reversal of the process. Immunologically, there is no apparent

TABLE I

Inhibitors of Herpes Simplex Virus Infection

| Chemical Nature | Specific Compounds | Mode of Action | Reference |
|---|---|---|---|
| 1) Halogen Pyrimidine Deoxyriboside | 5-iodo-2′-deoxy-uridine | Replaces thymidine in DNA; may inhibit enzymes related to DNA synthesis | (31) |
| 2) Pyrimidine Arabinoside | cytosine arabinoside | Inhibits synthesis of deoxycytidylic acid | (32) |
| 3) Anti-HSV antibodies | γ-globulins | Passive immunity; neutralize virus | (37) |
| 4) ds-Ribonucleic acid | Poly (I)·Poly (C) | Interferon inducer | (39) |
| 5) Steroids | prednisone, dexamethasone | Immunosuppression (?) | (38) |

reason why the host immune responses should not destroy the infection. Persons afflicted with recurrent herpes simplex infection usually have high levels of circulating anti-herpes antibody directed both against the virus (7) and the infected cell surface (personal observation). These should completely eradicate the infection. This situation is similar to experimental and clinical manifestations of the immune response to neoplastic disease. Cancer patients frequently have circulating antibody, and lymphocyte toxicity, directed against their tumors (43). These patients, however, also frequently have high levels of serum activity which blocks the effectiveness of the immune response against the tumor (44). A similar serum factor might be preventing anti-herpes antibody from reacting with infected cell surfaces. The role of serum-blocking activity in the immunology of latent and acute herpes simplex infection awaits further characterization of the blocking entity itself, and how it works to block eradication of neoplastic disease.

Also immunologically, the rediscovery of BCG (tubercle bacillus), especially as it relates to immunochemotherapy of skin cancer and metastatic neoplasia, (45, 46) may prove useful in the management of cutaneous herpes simplex infection. BCG, applied topically to the affected site, mobilizes the cell-mediated response to the foreign antigen by drawing immunocompetent cells (probably monocytes and macrophages) into the area. These cells, in conjunction with serum factors and induced hypersensitivity reactions, initiate the immune destruction of the skin tumors (47). The mobilization of immune responses in this way may also facilitate destruction of herpes simplex virus and infected cells.

Recent studies with colchicine and vinblastin indicate that these drugs may be active in inhibiting cell adhesion (48) and intracellular transport of synthesized materials (49). During the replication of herpes simplex virus, virus-specific materials are transported first, from the cytoplasm to the nucleus and then, assembled virus, from the nucleus to the cytoplasm to the outside of the cell. Preliminary experiments (personal observation) indicate that these drugs may be active in inhibiting intracellular transport of virus specific materials, blocking virus production.

In summary, although herpes simplex virus infections have proven very resistant to immunochemotherapy, the prognosis for future successful treatment is encouraging. New experimental approaches leading to an understanding of why the immune system sometimes fails to interact with antigenic foreign material will possibly explain how herpes simplex infection survives so successfully in an afflicted individual. Once this is explained, new immunologic approaches to the eradication of the infection can be attempted.

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# Tracheal Stenosis Secondary to Cuffed Tubes

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The present treatment of respiratory failure often employing endotracheal anesthesia with cuffed endotracheal and cuffed tracheostomy tubes may produce serious sequelae in a significant number of cases (1, 2). These disabling complications comprise tracheal erosion, tracheomalacia, and tracheal stenosis.

Tracheostomy tubes without cuffs may cause minor damage at the stoma consisting of minimal luminal compromise associated with mucosal edema and granulation tissue (3). These usually heal after decannulation and the site of the tracheostomy can be recognized roentgenologically as an anterior dimple. More extensive cartilage necrosis, especially after protracted tracheostomy, may lead to significant tracheal collapse. An endotracheal tube may cause inflammatory granulomas, ulcerations, and resultant adhesions at the unyielding sites of contact of the upper respiratory tract—namely, the arytenoids, the epiglottis, and the subglottic region.

The advent and use of the cuffed endotracheal and cuffed tracheostomy tubes for intermittent positive pressure ventilation have resulted in more serious lesions at the site of the cuff (4, 5). These lesions are being recognized more frequently with increasing use of this treatment and are the subject of this report.

## *Etiology*

The purpose of the cuff is to provide an airtight seal of the airway system so that positive pressure ventilation proceeds through the tube only. On the average, four to ten ml of air, when injected into the cuff, will provide an adequate seal. This minimal occluding volume will vary with the type of tube, size of the cuff employed, ventilatory pressure required, and the tracheal diameter. The precise safe volume of air is difficult to determine in any individual case. With the means now available it is impossible to totally avoid complications. Some cuffs do not empty readily and completely. Periodic deflation and reinflation of the cuff has been recommended in an attempt to minimize local pressure effects, but requires caution. Overinflation of the cuff may result when the minimal occluding volume is added to a volume of residual air.

The frequency and severity of tracheal mucosal injury is directly proportional to the degree of pressure exerted at the site of the inflated cuff and the length of time this pressure is maintained (6). Tracheal injury is observed in all patients with indwelling cuffs. However, this injury is usually mild, consisting of mucosal edema and superficial erosion. The resultant healing leaves no disabling

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deformity. It is difficult to estimate the incidence of symptomatic stenosis at the level of the cuff since many of the patients requiring controlled respiration succumb to their disease (7). However in those that do survive, the occurrence of tracheal stenosis at a later date is about fifteen percent. In most series, the minimal time of cuffed tube intubation before stenosis occurs was forty-eight hours (8, 9).

### *Pathogenesis and Pathology*

The cuff injury presumably results from progressive ischemic pressure necrosis of the mucosa over the unyielding cartilaginous rings. The blood supply of the trachea is derived primarily from the inferior thyroid artery which sends branches transversely in the fibromuscular intercartilaginous membrane (10). Vertical channels connect the horizontal vessels. These vertical vessels, interposed between the rigid cartilage externally and the mucosal surface internally, are especially vulnerable to early occlusion by the inflated cuff. Eventually, with sustained pressure, the transverse arteries in the intercartilaginous membrane may also be compressed. For these reasons, ischemia initially involves the antero-lateral two-thirds of the tracheal mucosa covering unyielding cartilage. The posterior membranous tracheal wall is corrugated and distensible. This common wall with the esophagus has an abundant vascular network both from tracheal and esophageal vessels. These vessels contained between pliable mucosal surfaces are less readily occluded by distension of the cuff. A posterior bulge into the esophageal lumen can be recognized endoscopically at the level of the inflated cuff. These anatomic features explain the less frequent or late involvement of the posterior tracheal wall in stenotic cuff lesions. With continuation of excessive pressure, necrosis of all layers of the wall, including the cartilage, can ensue.

In addition to direct pressure from the cuff, some observers have also implicated shearing forces between the trachea and the cuff resulting from the piston-like movement of the tube imparted by the intermittent positive pressure apparatus (11). In some cases, secondary infection may play a significant role (12).

Pathologically, the fully developed lesions reveal total replacement of the mucosa, submucous glands, and cartilage by granulation tissue of varying maturity (13). Adjacent to the zone of maximal injury, lesser degrees of damage are seen, such as erosion and fragmentation of the cartilage, marked inflammatory infiltration, ulceration of the mucosa, and areas of squamous metaplastic reparative attempts (Fig. 1).

After extubation, healing may lead to cicatricial stenosis. At least fifty percent of the stenotic lesions spare the posterior membranous wall. These stenoses on transverse section have a triangular configuration by virtue of the fact that weakening of the cartilaginous support anteriorly results in lateral collapse of the tracheal wall (Fig. 2). In advanced cases in which the posterior wall is also involved, circumferential stenosis may result (Fig. 3).

*Clinical Features*

The length of time between extubation and the diagnosis of the stenosis has varied from two weeks to as long as one and one-half years. Most cases come to attention within six weeks of decannulation or extubation (14). When the

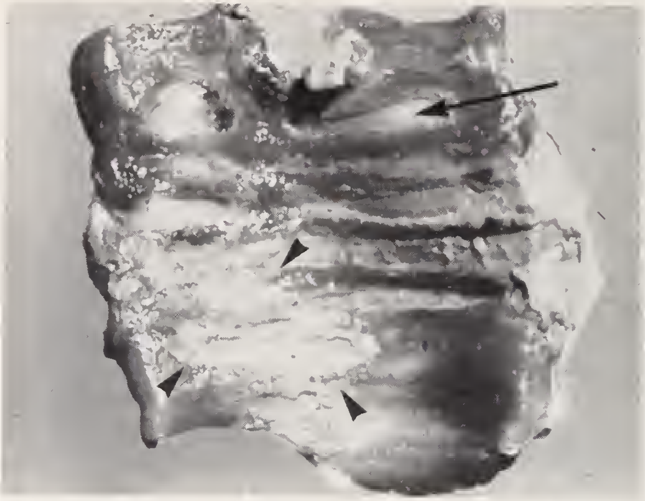

FIG. 1. Postmortem specimen of trachea opened posteriorly showing varying degrees of tracheal injury from a cuff. The tracheostomy opening is seen at the upper end of the specimen. A large, irregular ulceration with granulation tissue at its margins and fragmented cartilage at its base is present (arrowheads) and represents the site of maximal cuff injury. The pale band-like areas (arrow) are exposed cartilage rings. The dark band-like areas between the exposed cartilages are due to intervening hyperemic mucosa.

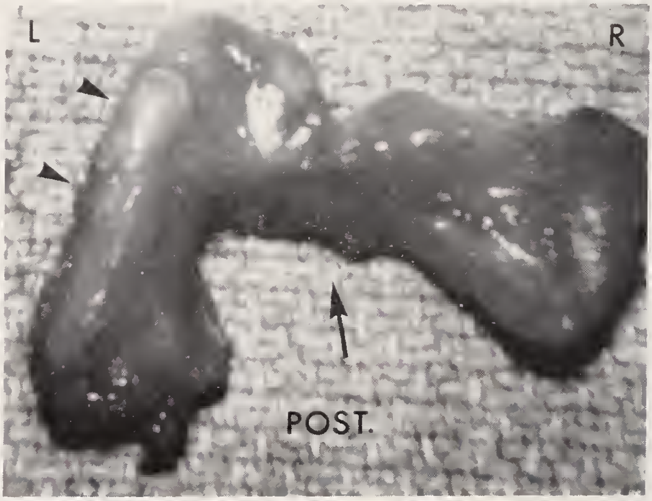

FIG. 2. Resected tracheal segment seen from above. The membranous posterior wall was not resected and was utilized for reconstructive surgery. Only a short piece of exposed residual cartilage is seen on the left (arrowheads). The remainder of the anterior wall is thickened and deformed with a fibromembranous, web-like protrusion extending into the lumen (arrow).

tracheal lumen is reduced to as little as three to four mm in diameter, stridor is the characteristic finding. Stridor or dyspnea, with wider tracheal lumen, may occur in stenoses which are relatively pliable. The lumen in these cases becomes further compromised during breathing due to collapse of the tracheal wall at the site where cartilaginous rings have been destroyed. In milder narrowings, dyspnea may become evident only on exertion. Some of these patients have been misdiagnosed as bronchial asthma (15). Reduction of seventy-five percent of the luminal area or fifty percent of the diameter of the trachea is compatible with normal exercise tolerance if the patient has normal lungs. The same defect may provide considerable disability in individuals with chronic lung disease. Such patients are unable effectively to raise tracheo-bronchial secretions beyond the stricture and they suffer recurrent pneumonias. If the patient presents with a clinical picture of severe respiratory distress with a tracheostomy tube in place, one must suspect either a long stenotic segment which extends beyond the endotracheal opening of the tracheostomy tube or a second stenotic segment distally.

### *Roentgenographic Features*

Roentgenologically, stenoses are investigated by routine examination of the neck and chest in antero-posterior, oblique, and lateral views. More specific modalities utilize high KV and high filtration techniques, tomography in the frontal and lateral projections, and contrast tracheograms. Care should be taken to examine the tracheal air column on all chest films obtained for evaluation of respiratory ailments since, not only can the stenosis be suggested, but additional information pertaining to other causes of the patient's symptoms may be discovered (16). The oblique projection helps to project the stenotic segment in the

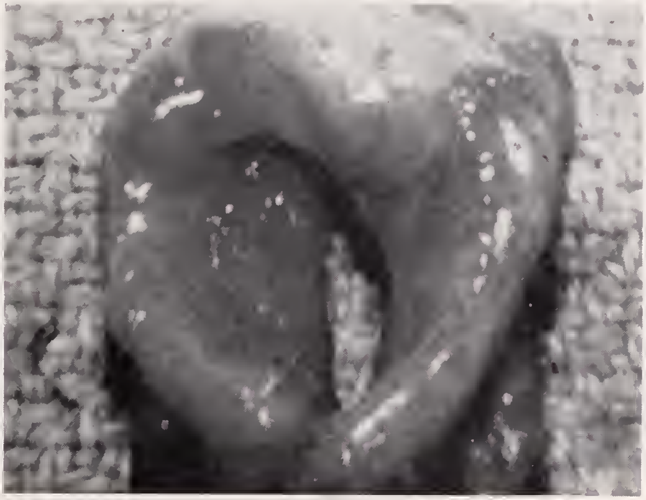

Fig. 3. Resected tracheal segment viewed from above. There are two-crescentic, valve-like stenotic areas with tapering margins clearly identified at different tracheal levels. These produce a slit-like lumen. Side-to-side collapse is a result of fragmentation and loss of the supporting cartilaginous rings.

mediastinal trachea off the spine. If the patient's examination reveals a tracheostomy tube in place, the tube should be removed if feasible. If the patient cannot tolerate tube removal, a plastic nonradiopaque tube can be inserted in place of the standard metal tube. In either case, information pertaining to the tube's position within the trachea and how completely it traverses the stenotic segment

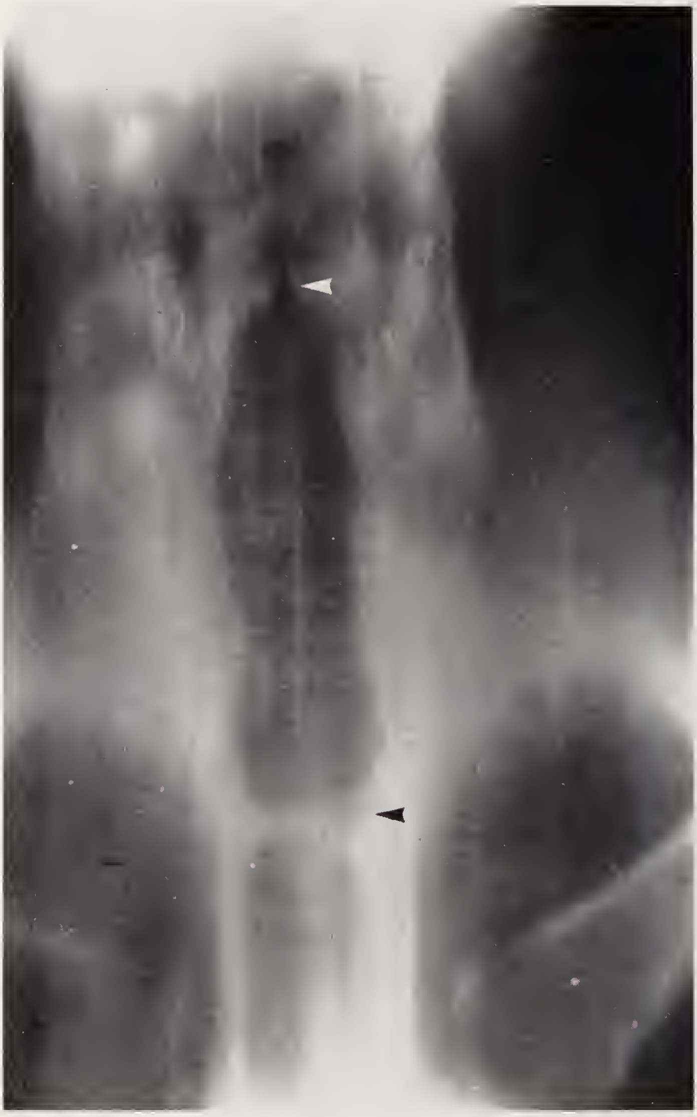

Fig. 4. Frontal tomogram of a patient with a thin, web-like stenosis (black arrowhead) at the root of the neck. The distance from the cords (white arrowhead) can be accurately determined. This type of stenosis usually involves only one tracheal ring and is not completely symmetrical. The edges are narrow and sharply sloping. The remaining tracheal diameter is normal.

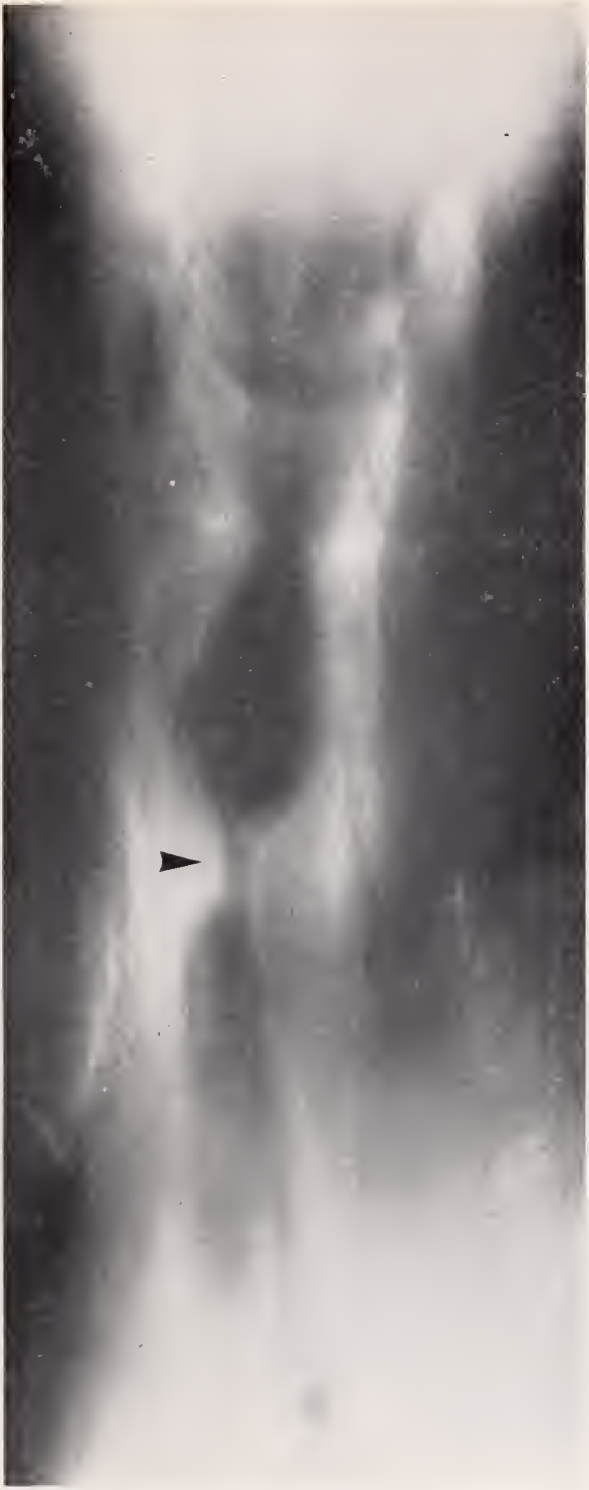

FIG. 5. Frontal tomogram of trachea showing a stenosis involving more than one tracheal ring at the root of the neck (arrowhead). The stenotic segment is long, with broadly tapering edges. There is marked narrowing of the lumen.

should be noted. A tracheal suction set-up should be available in the examination room (17).

A contrast media tracheogram clearly delineates the length and shape of the stenosis and often demonstrates that the stenosis is more extensive than sus-

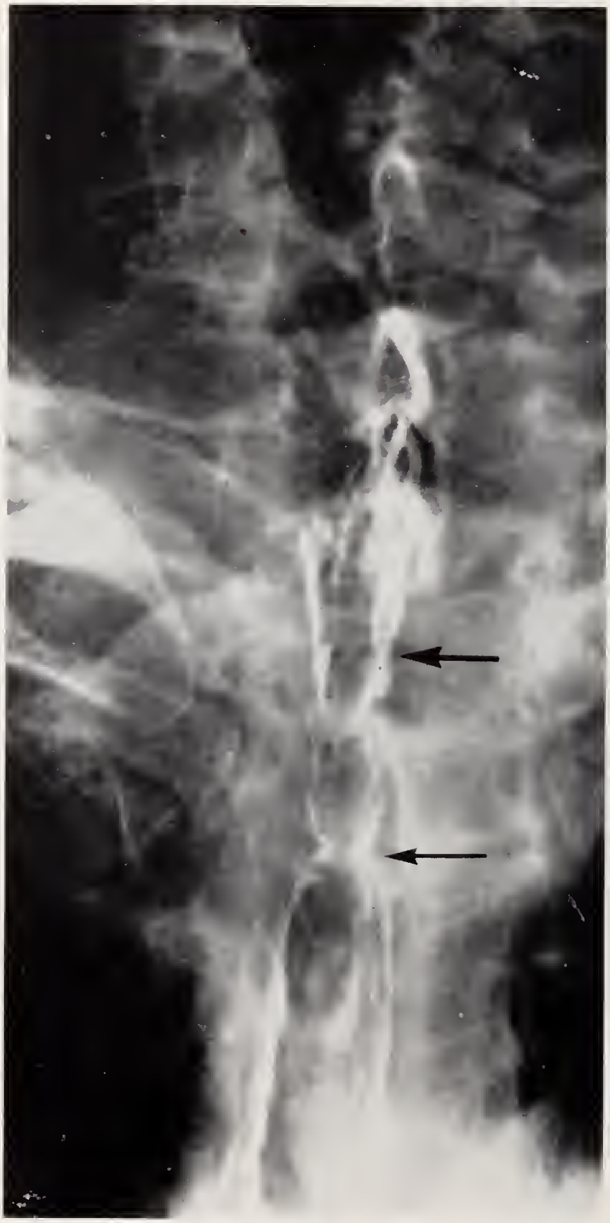

FIG. 6. Frontal film from Dionosil® study showing a long segment of stenosis (between arrows) just below the level of the clavicles. The normal tracheal diameter is seen just below the stenosis. This segment did not change with respiration.

pected on air contrast films. The opaque material can be introduced via the larynx, or if a tracheostomy stoma is present, via the tracheostomy opening.

In extreme luminal compromise, it is suggested that positive contrast tracheography may be hazardous. However, in our experience adverse reactions to contrast media were not observed. This modality has provided additional information not obtained by other roentgenographic examinations. If contrast material is to be employed, oily Dionosil® is the current agent of choice.

If only one tracheal cartilage is involved, the resulting stenosis is usually thin, appearing as a web. It is seen both in the frontal and oblique projections and can often be missed on the lateral film (Fig. 4). The web encompasses the trachea anterolaterally while the posterior wall is spared. On the other hand, when two to five tracheal rings have been destroyed, the stenotic segment is

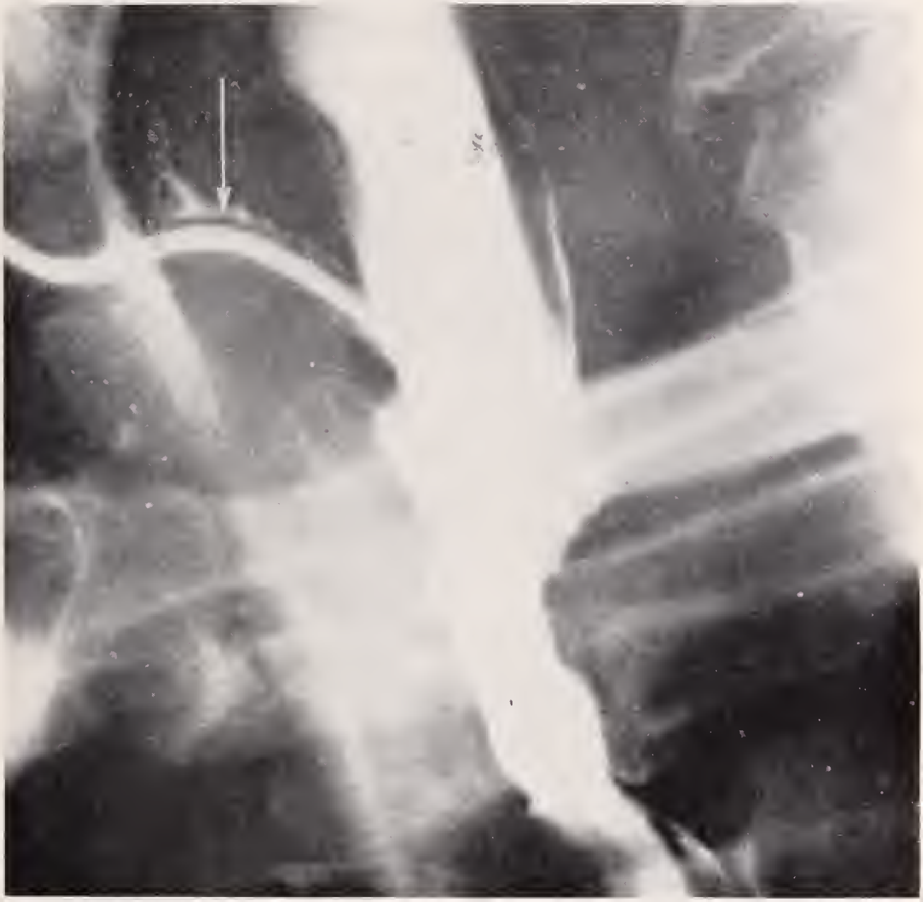

Fig. 7. Oblique film from Dionosil study showing extensive tracheal damage due to cuffed tubes and multiple tracheostomies. Contrast material outlines the soft rubber tube (arrow) which was passed through the tracheostome located just above the clavicles. The cervical portion of the trachea above the tracheostome is widened, "saucerized," and flaccid. The thoracic portion is irregularly narrowed and rigid.

longer and larger, somewhat complex, and has tapering margins (Fig. 5). If the narrowed segment is pliable, the resulting collapse with inspiratory effort can produce luminal narrowing out of proportion to the actual degree of luminal compromise and can be seriously disabling. This element of tracheomalacia is best assessed dynamically by fluoroscopy or cineradiography. By contrast, if the stenosis is rigid, it will retain its shape during respiratory excursions. In patients with posterior tracheal wall involvement, the resulting stenosis is irregularly circular, often elongated with marked luminal compromise. The stenosis is easily recognized in all projections (Figs. 6–10).

Roentgenographic studies localize the stenosis to the cervical or mediastinal part of the trachea. They define the distance between the vocal cords and the stenosis and the distance between the stenosis and the tracheostomy stoma and the carina. The stenotic segment is frequently one or two cm below the tracheostomy stoma (18). Depending upon the exact site of the tracheostomy, the

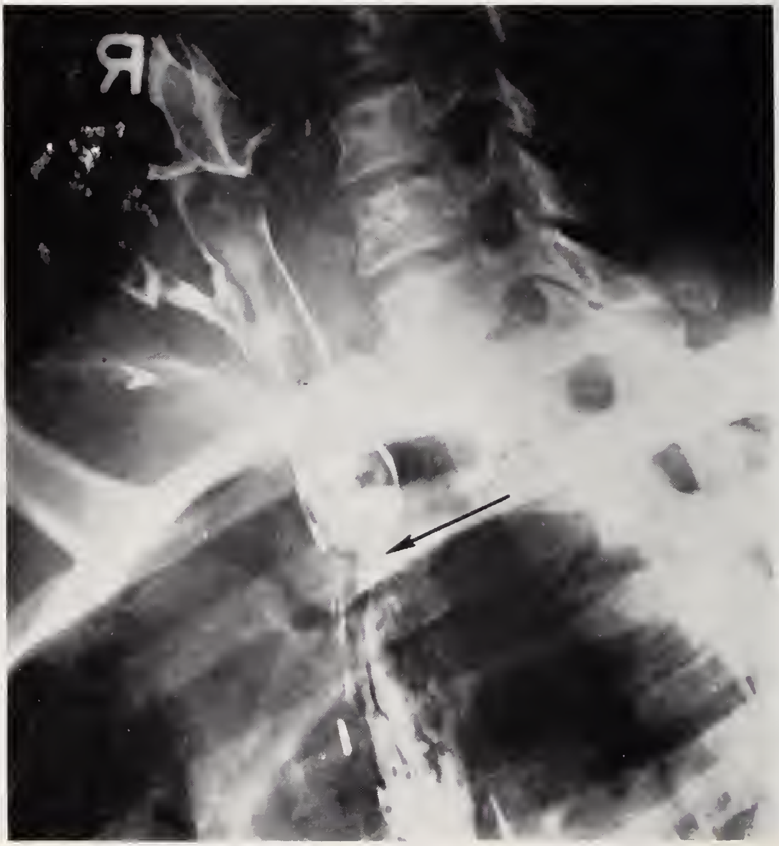

Fig. 8. Oblique projection of patient with a long history of cardiac disease eventually requiring cardiac surgery. She needed positive pressure ventilation for two weeks postoperatively. The resulting tracheal stenosis (arrow) is at the level of the clavicles. The stenosis is severe, with marked tracheal narrowing and broad, sloping edges. The tracheostomy is also outlined by contrast material.

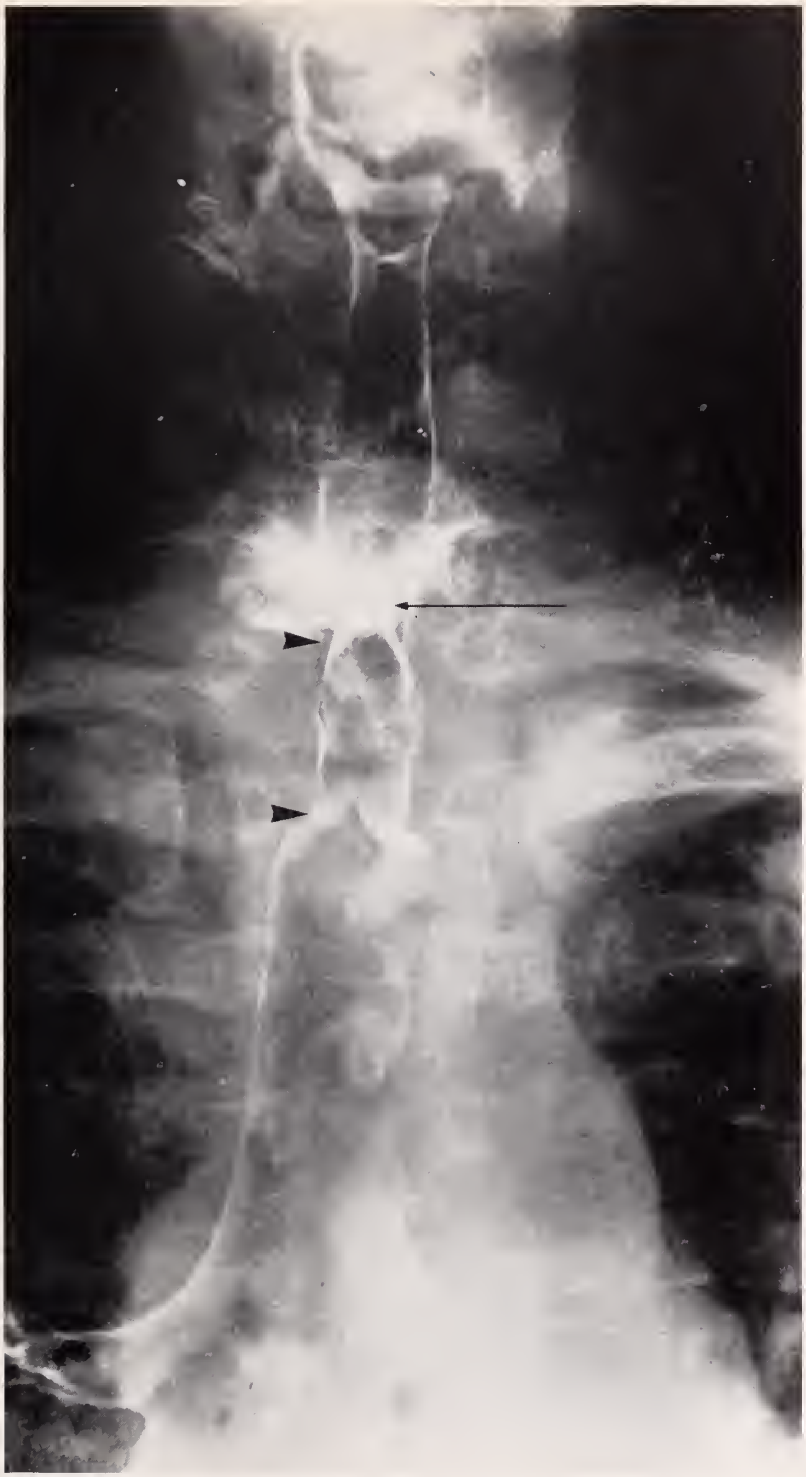

Fig. 9. Frontal film from a Dionosil study showing a long, severe stenosis (between arrowheads) at the level of the clavicles. The tracheostomy stoma (arrow) is located just above the stenotic region. A variety of cuffed tubes was employed over a period of years.

length of the tube and the size of the tube cuff, the stenosis can be located in the middle or lower cervical region. This information is essential to the surgeon's selection of the proper size tracheostomy tube for conservative management or for his surgical approach.

Multiple stenoses occur especially when frequent deflation and inflation of the cuff have been practiced and where tubes of varying lengths and tubes with double cuffs have been employed (Fig. 11). The second stenosis may be located at the mediastinal level and require a mediastinal exploration with a con-

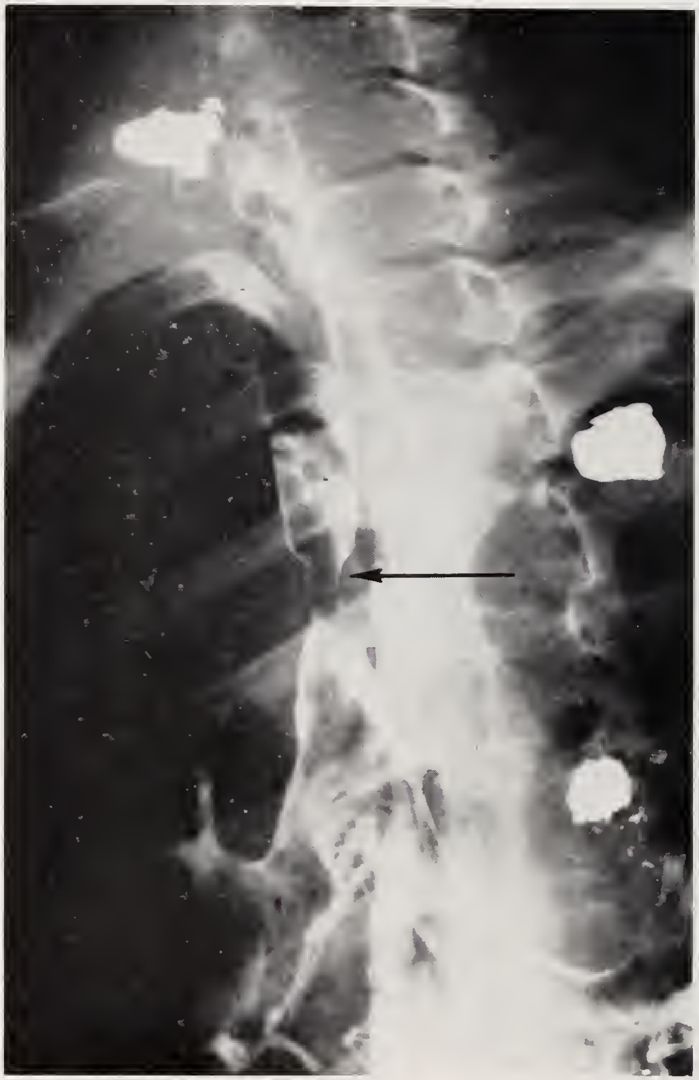

Fig. 10. Oblique view from a Dionosil study showing a stenosis (arrow) of the thoracic trachea. The patient required assisted ventilation after being shot (bullets identified on film). The stenosis was not well visualized without contrast material. It encircles the trachea and extends over several tracheal rings.

sequently less favorable prognosis. Coincidental stenosis at the site of the tracheostomy is not infrequent.

Conservative therapy consisting of dilatation has proved to be ineffective in most cases (13, 19–21). Segmental resection of the stenotic lesion with end-to-end tracheal anastomosis is the treatment of choice (22). Tracheal resection is more easily performed in the neck than in the mediastinum. Accordingly, tra-

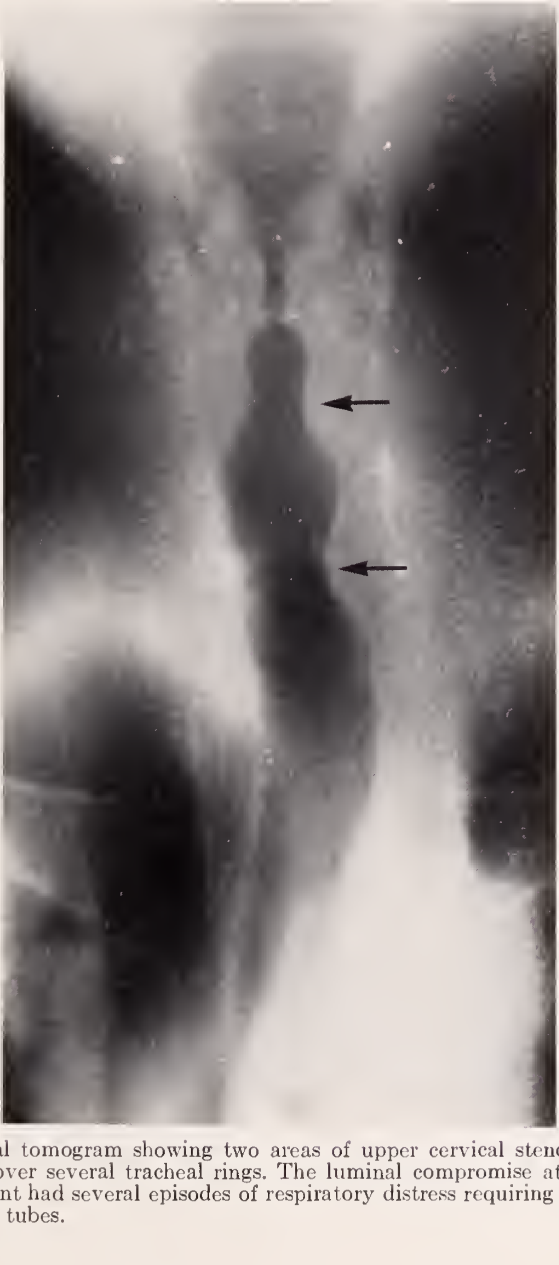

FIG. 11. Frontal tomogram showing two areas of upper cervical stenosis (arrows). Each stenosis extends over several tracheal rings. The luminal compromise at both levels is not severe. This patient had several episodes of respiratory distress requiring assisted ventilation with different size tubes.

cheostomies should preferably be done at a higher rather than a lower cervical level. Should stenosis occur, a cervical approach would then be feasible.

## Conclusion

Tracheal stenosis in patients in whom cuffed tubes have been employed is not a rare occurrence and results from pressure necrosis. These lesions are best evaluated radiographically and can be visualized either as thin diaphragms or as stenotic segments involving two to five tracheal cartilages. Roentgen studies, in combination with endoscopic examination where indicated, provide the precise localization and delineation of the stenotic segment or segments required for proper surgical correction.

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# Newer Concepts of Pancreatic Secretory Patterns.

## Pancreatic Secretory Mass and Pancreatic Secretory Capacity: Pancreatic Hypersecretion

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In the classical studies with secretin, normal ranges for the pancreatic responses of flow, bicarbonate, and amylase were calculated from the data of patients without pancreatic disease (1) (Table I). Based upon these ranges, various patterns of pancreatic secretory deficiency could be discerned in patients with pancreatic cancer and pancreatic inflammatory disease (2, 3) (Table II). The pattern of discordant secretion or hypersecretion, described almost 25 years ago for patients with cirrhosis, was poorly understood, probably because attention was focused on the deficiency states.

This study analyzes the secretory data of a large series of patients with "pancreatic hypersecretion"—Zollinger-Ellison syndrome; cirrhosis; hemachromatosis; and chronic pancreatitis—and presents the hypothesis that under suitable stimulation the pancreatic ductular mass and hence its secretory capacity can be greatly augmented by hyperplasia and hypertrophy.

### Method

The secretory data was obtained from a standard secretin test using 1.0 $\mu$/Kg immediately followed by an augmented test using 4.0–5.0 $\mu$/Kg according to the technique described by the pancreatic laboratory. The normal ranges for pancreatic responses are as follows:

| | |
|---|---|
| 1. Flow | |
| a) Mean | 259 ml/80′- |
| b) Upper limit | 395 ml/80′- |
| 2. $HCO_3$ Concentration | |
| a) Mean | 90 mEq |
| b) Upper limit | 130 mEq |
| 3. $HCO_3$ Concentration | |
| a) Mean | 26.8 mEq/80′ |
| b) Upper limit | 35.6 mEq/80′ |

*Zollinger-Ellison Cases*

Interest in Zollinger-Ellison patients was generated by a report of Dyck (4) of a patient with this disorder who showed functional hypersecretion following secretin. The physiologic implication of this "functional hypersecretion" would be the neutralization of excessive gastric acid secretion by the enormous

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quantities of pancreatic bicarbonate which, of course, would not only protect against ulceration, but also facilitate digestion and absorption. Accordingly, in this group of patients the following parameters were studied:

1. Basal gastric secretion
2. Augmented gastric secretion
3. Basal duodenal secretion
4. Standard pancreatic secretion
5. Augmented pancreatic secretion

The basal and augmented gastric secretory data are summarized in Table III.

TABLE I

*Means and Ranges as Calculated from Data of 123 Patients without Pancreatic Disease*

| | 80′ Flow | | $HCO_3$ | |
|---|---|---|---|---|
| | ml | ml/Kg | mEq/L | mEq/80′ |
| Mean | 200 | 3.2 | 108 | 20.8 |
| Lower limit | 128 | 2.0 | 90 | 16.8 |
| Upper limit | 272 | 4.0 | 130 | 24.9 |

TABLE II

*Patterns of Pancreatic Secretion*

| | |
|---|---|
| 1. Total deficiency | —Cancer or calcinosis |
| 2. Quantitative deficiency | Cancer |
| 3. Qualitative deficiency | —Pancreatitis |
| 4. Isolated enzyme deficiency | —Nutritional pancreatitis |
| 5. Discordant secretion | —Hypersecretion |

TABLE III

*Basal and Augmented Gastric Secretory Data of Ten Patients with Zollinger-Ellison Syndrome*

| | Basal-Unstimulated | | Histamine-Stimulated | | |
|---|---|---|---|---|---|
| | Flow ml/hr | Acid content mEq/hr | Flow ml/hr | Acid content mEq/hr | |
| 1 | 365 | 41.3 | 432 | 52.8 | o |
| 2 | 218 | 27.8 | 411 | 56.6 | o |
| 3 | 316 | 44.1 | 475 | 54.0 | o |
| 4 | 426 | 31.5 | 406 | 48.0 | o |
| 5 | 391 | 32.7 | 478 | 59.5 | x |
| 6 | 407 | 28.5 | 499 | 60.1 | x |
| 7 | 283 | 43.4 | 475 | 53.3 | x |
| 8 | 515 | 60.1 | 603 | 68.4 | x |
| 9 | 602 | 74.3 | 393 | 54.6 | x |
| 10 | 527 | 58.7 | 461 | 67.4 | x |
| Average | 405 | 44.3 | 463 | 57.5 | |

The data illustrate the high rates of unstimulated gastric acid secretion (Average: 44.3 mEq/hr) and also the minimal effect of a maximal histamine stimulus on this rate (Average: 57.5 mEq/hr). Comparable pancreatic secretory data is given in Table IV. Not only are the basal flows and bicarbonate secretion greatly increased in over half the patients, but it is clear that the average standard secretin bicarbonate secretion, 46.3 mEq/hr, and certainly the average augmented secretin bicarbonate secretion, 99.7 mEq/hr, are sufficient to neutralize the gastric acid flows observed in these patients. To emphasize these physiologic observations, data comparing gastric secretory findings in various diseased states are presented in Table V; data comparing pancreatic secretory findings

TABLE IV

*Basal and Secretin-Stimulated Pancreatic Secretion in Patients with Zollinger-Ellison Syndrome*

| Patients | Basal-Unstimulated | | | Secretin-Stimulated | | | |
|---|---|---|---|---|---|---|---|
| | | | | Standard | | Augmented | |
| | Duodenal pH | Flow ml/hr | $HCO_3$ content mEq/hr | Flow ml/hr | $HCO_3$ content mEq/hr | Flow ml/hr | $HCO_3$ content mEq/hr |
| 1 | 1.3 | 198 | | 492 | 52.6 | | |
| 2 | 1.5 | 45 | | 298 | 30.1 | | |
| 3 | 7.0 | 99 | 1.6 | 318 | 30.8 | | |
| 4 | 7.2 | 84 | 8.7 | 355 | 36.9 | | |
| 5 | 1.7 | 170 | | 692 | 63.7 | | |
| 6 | 2.1 | 240 | | 486 | 36.7 | | |
| 7 | 3.0 | 280 | | 235 | 22.0 | 528 | 58.5 |
| 8 | 8.0 | 230 | 46.0 | 390 | 50.1 | 785 | 98.4 |
| 9 | 7.4 | 456 | 54.7 | 779 | 72.9 | 1340 | 132.1 |
| 10 | 7.8 | 186 | 22.3 | 670 | 66.8 | 1131 | 109.7 |
| Average | | 199 | 36.3 | 471 | 46.3 | 946 | 99.7 |

TABLE V

*Gastric Secretory Data of Normal Subjects, Patients with Gastric and Duodenal Ulcer, Patients with Hemachromatosis, and Patients with Zollinger-Ellison Syndrome*

| | # Patients | Basal Aver. pH | Basal Aver. Flow ml/hr | Basal Aver. Flow mEq/hr | Augmented Aver. Acid mEq/hr |
|---|---|---|---|---|---|
| Normals | 350 | 5.3 | 66 | 2.3 | 28.7 |
| Alc. Panc. | 154 | 5.1 | 72 | | |
| Bil. Panc. | 78 | 5.5 | 66 | | |
| G.U. | 22 | 4.9 | 60 | 2.7 | |
| D.U. | 55 | 1.5 | 152 | 8.5 | 35.9 |
| Z.E. | 10 | 1.0 | 405 | 44.3 | 57.5 |
| Hemachromatosis | 18 | 5.0 | 88 | 1.8 | 25.3 |

in patients without pancreatic disease, with Zollinger-Ellison syndrome, and with pancreatic hypersecretion, are summarized in Table VI, and data comparing acid and bicarbonate outputs are given in Table VII.

The interpretation (5) of this data would be that in Zollinger-Ellison syndrome, gastric hypersecretion is associated with a pancreatic hypersecretion of fluid and bicarbonate resulting from the action of increased releases of endogenous secretin in the duodenum upon a pancreatic ductular mass which has been augmented, presumably by hyperplasia and hypertrophy, in an attempt by the organism to maintain duodenal homeostasis.

### *Cirrhoses*

The demonstration of pancreatic hypersecretion in Zollinger-Ellison patients and the hypothesis of increased ductular mass prompted study of other possible states of pancreatic hyperfunction and required more precise elucidation of the origin of the increased flows. The grouped data of all our patients with pancreatic hypersecretion are given in Table VIII. The patients suffer from: biliary cirrhosis; postnecrotic nonalcoholic cirrhosis; alcoholic cirrhosis; Zollinger-

TABLE VI

*Pancreatic Secretory Data of Normal Patients, Patients with Zollinger-Ellison Syndrome, and Patients with Hemachromatosis*

| | Patients | Basal-Unstimulated | | Secretin-Stimulated | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Standard | | Augmented | | |
| | | Flow ml/hr | $HCO_3$ content mEq/hr | Flow ml/hr | $HCO_3$ content mEq/hr | Flow ml/hr | $HCO_3$ content mEq/hr | |
| Normals | 172 | 42 | 10.5 | 254 | 25.5 | 413 | 41.7 | * |
| Z.E. | 10 | 199 | 36.6 | 471 | 46.3 | 946 | 99.7 | ⁰ |
| Hemachromatosis | 18 | 88 | 15.7 | 761 | 61.3 | 1131 | 119.8 | + |

TABLE VII

*Comparison of Acid Output and Bicarbonate Secretory Rates in Normal Subjects, in Patients with Duodenal Ulcer, and in Patients with Zollinger-Ellison Syndrome*

| | Aver. Peak Acid Output mEq/h | Aver. Standard Secretin $HCO_3$ Content mEq/hr | Aver. Augmented Secretin $HCO_3$ Content mEq/hr |
|---|---|---|---|
| Normals | 24.6 | 34.2 | 44.7 |
| D.U. | 35.9 | 30.0 | 56.8 |
| Z.E. | 57.5 | 46.3 | 99.7 |

TABLE VIII

*Pancreatic Flow and Bicarbonate Response Data for the Standard and Augmented Test in Patients with Pancreatic Hypersecretion*

Post Secretion Data—Pancreatic Hypersecreation

| | Standard 80 min. | | | Augmented 80 min. | | |
|---|---|---|---|---|---|---|
| | * | Ave. flow | Ave. $HCO_3$ content | * | Ave. flow | Ave. $HCO_3$ content |
| | | ml. | mEq. | | ml. | mEq. |
| Biliary cirrhosis | 44 | 294 | 32.4 | 1 | 587 | 50.3 |
| Nonalcoholic cirrhosis | 39 | 307 | 36.5 | 2 | 591 | 58.4 |
| Alcoholic cirrhosis | 95 | 401 | 43.2 | 3 | 874 | 76.6 |
| Zollinger-Ellison | 11 | 483 | 48.5 | 5 | 911 | 97.9 |
| Hemachromatosis | 19 | 772 | 60.9 | 3 | 1249 | 118.5 |
| Normal average range | 257 | 259 | 26.8 | 22 | 438 | 44.7 |
| | | 117–395 | 15.9–35.6 | | 248–570 | 28.5–57.9 |

Ellison syndrome, and hemachromatosis. Analysis of this data indicates three patterns of secretion:

1. Biliary cirrhosis and nonalcoholic cirrhosis in which the flows and bicarbonate secretion are elevated well above the average for normals but do not fall outside the normal ranges;
2. Zollinger-Ellison and hemachromatosis patients in whom the average flow and secretory rates are significantly above normal ranges; and
3. The secretory data of patients with alcoholic cirrhosis which fall between these two groups.

The interpretation of these data (6) requires definition of what is meant by "pancreatic hypersecretion" as well as elucidation of the sources of fluid collected from the duodenum following secretin stimulation.

"Hypersecretion" of the pancreas implies a flow or bicarbonate response to secretin stimulation in excess of two standard deviations above the mean calculated from the data of normal patients. Duodenal fluid collections after secretin consist of pancreatic juice, duodenal juice, and a contribution of a bicarbonate bile produced by the hepatic ductular system in response to secretin. In seven patients with complete obstructive jaundice, external biliary fistulas have yielded following secretin an average flow of 45 ml/hr and an average bicarbonate content of 3.4 mEq/hr.

Considering these facts, it is doubtful whether the term pancreatic hypersecretory state should be applied to patients with biliary cirrhosis and nonalcoholic cirrhosis. Hepatic ductular reduplication well recognized in these pathologies would provide sufficient secretin-responsive parenchyma to explain the average upper limit normal range flows, that is, 294 ml and 307 ml/80 as well as the average upper limit bicarbonate secretions, that is, 30.4 mEq and 36.5 mEq/80 min observed in these patients. Moreover pathologic involvement of the pancreas is absent both in biliary cirrhosis and nonalcoholic cirrhosis.

Interpretation of the pattern of secretion observed in patients with hemachromatosis justifies the label pancreatic hypersecretion. The postsecretin flows, that is, 483 ml and 772 ml/80 min, are significantly above the 2 sigma level and the postsecretin bicarbonate contents, that is, 48.5 and 50.9 mEq, almost double the upper limit of bicarbonate secretion of controls in response to standard testing with secretin. In patients with Zollinger-Ellison syndrome, there is no physiologic reason and no histologic evidence of hepatic ductular pathology which might indicate that the increased flow and increased bicarbonate secretion derives from the liver. In patients with hemachromatosis some hepatic contribution to the enormous postsecretin volumes and postsecretin bicarbonate must be accepted from the known participation of the liver in this disease, but the order of magnitude of the augmentation of flow (500 ml/80 min) and bicarbonate secretion (30 mEq/80 min) is well above the highest bile flows and bicarbonate secretion, that is, 120 ml/80 min and 9.2 mEq/80 min, that have been observed in the pancreatic laboratory following 1 $\mu$/Kg secretin. Further, examination of the Zollinger-Ellison and hemachromatosis data reveals that these patients are responding to a standard dose of secretin at rates of flow comparable to those obtained following the augumented dose of 4.0 $\mu$/Kg in control patients. Such a secretory pattern would imply either: (1) increased sensitivity of the ductular parenchyma to secretin; (2) increased ductular cell mass and hence increased secretory capacity; or (3) both increased parenchymal sensitivity and mass. While the possibility exists in patients with Zollinger-Ellison syndrome that the background level of increased gastrin secretion might, by synergistic action with secretin, increase the pancreatic responsiveness to the hormone, no such mechanism exists to explain the huge flow and bicarbonate data observed in patients with hemachromatosis. The conclusion seems warranted that the evidence in patients with Zollinger-Ellison syndrome would support "functional hypersecretion" at least, while in hemachromatosis parenchymal augmentation is necessary to account for the pancreatorrhea.

The data of patients with alcoholic cirrhosis might be expected, as it does, to fall midway between the findings in the postulated biliary augmented flows and bicarbonate pattern of secretion of patients with biliary and nonalcoholic cirrhosis and the postulated biliary, augmented and pancreatic augmented flows and bicarbonate patterns of patients with Zollinger-Ellison and hemachromatosis. It is generally accepted that the alcoholic cirrhotic almost universally exhibits minimal areas of pancreatic fibrosis though rarely manifests clinical pancreatitis. While it might be argued by some that such "minimal pancreatitis" might decrease the pancreatic flow and bicarbonate response to secretin, evidence can be presented that this minimal fibrosis is associated with ductular reduplication and that such minimal lesions in alcoholics with clinically manifest pancreatitis are associated with increased pancreatic flow.

Analysis of the data of patients with pancreatic hypersecretion warrants four conclusions (6):

1. Patients with cirrhosis, Zollinger-Ellison syndrome and with hemachromatosis exhibit high rates of flow and bicarbonate secretion.

2. In patients with biliary cirrhosis and nonalcoholic cirrhosis, these increased secretory rates are interpreted to derive from an increased ductular cell mass in the liver.
3. In patients with Zollinger-Ellison syndrome and hemachromatosis, the hypersecretion is pancreatic, perhaps "functional" in the Zollinger-Ellison patient but resulting from increased pancreatic ductular mass in patients with hemachromatosis.
4. In patients with alcoholic cirrhosis, the increased flows and bicarbonate are interpreted as deriving from ductular reduplication in both the pancreas and liver.

### *Pancreatic Regeneration and Ductular Mass*

The observation that in alcoholic cirrhosis pancreatic flow and bicarbonate is increased, while in alcholic pancreatitis diminished secretion is seen, requires elucidation. Table IX presents the secretin responses of patients with pancreatic hypersecretion and summarized data of a group of alcoholics in whom there was no clinical nor laboratory evidence of either cirrhosis or pancreatitis. The patients with uncomplicated alcoholism fall in the hypersecretion range. On the other hand, when the data of patients with alcoholic pancreatitis are carefully scrutinized (Table X) it is apparent that decreased flow and decreased bicarbonate are only the average response. In this group, as well as in patients with biliary tract associated pancreatitis, there is a high percentage of patients in the hypersecretion range for flow. These unexpected findings are emphasized by frequency distribution plots of flow and bicarbonate secretion.

It is obvious that patients with chronic alcoholic and biliary tract pancreatitis display a two-population distribution with regard to flow but a normal distribution in regard to bicarbonate secretion (Fig. 1). The following hypothesis is presented to explain these observations: The initial response of the pancreas to injury, as in the liver, is a process of regeneration, that is, pancreatic ductular reduplication, hypertrophy, and/or hyperplasia. Such a concept would account for the increased average flows observed in patients with uncomplicated alcoholism and also would explain the frequent observation of high flow seen in chronic

TABLE IX

*Postsecretin Data of Patients with Uncomplicated Alcoholism and with Pancreatic Hypersecretion*

Post Secretin Data Pancreatic "Hypersecretion"

| | # | Ave. 80′ Flow ml/Kg | Ave. Max $HCO_3$ mEq/L | Ave. 80′ $HCO_3$ Secretion mEq |
|---|---|---|---|---|
| Alcoholism | 28 | 4.9 | 95 | 30.6 |
| Biliary cirrhosis | 44 | 4.4 | 108 | 32.4 |
| Nonalcoholic cirrhosis | 39 | 4.5 | 106 | 36.5 |
| Alcoholic cirrhosis | 95 | 6.2 | 94 | 43.2 |
| Hemachromatosis | 19 | 9.4 | 96 | 60.9 |

pancreatitis. As the inflammation progresses, ductular reduplication is replaced by fibrosis which is manifested by diminution in bicarbonate concentration. Finally, additional parenchymal replacement and fibrosis leads to the lower flows, low bicarbonate secretions which are characteristic of chronic pancreatitis.

TABLE X

*Postsecretin Data of Patients with Chronic Pancreatitis*

| | # | Ave. Flow ml/Kg/80 min | Ave. Max $HCO_3$ Conc. mEq/L | Ave. $HCO_3$ Secretion mEq/80 min |
|---|---|---|---|---|
| Alcoholic pancreatitis | 204 | 2.6 | 56 | 6.4 |
| % above lower limit | | 67% | 2% | 12% |
| % above upper limit | | 14% | 0% | 0% |
| Biliary tract pancreatitis | 104 | 2.9 | 64 | 7.8 |
| % above lower limit | | 75% | 2% | 14% |
| % above upper limit | | 21% | 0% | 0% |
| Alcoholism | 28 | 4.9 | 95 | 30.6 |

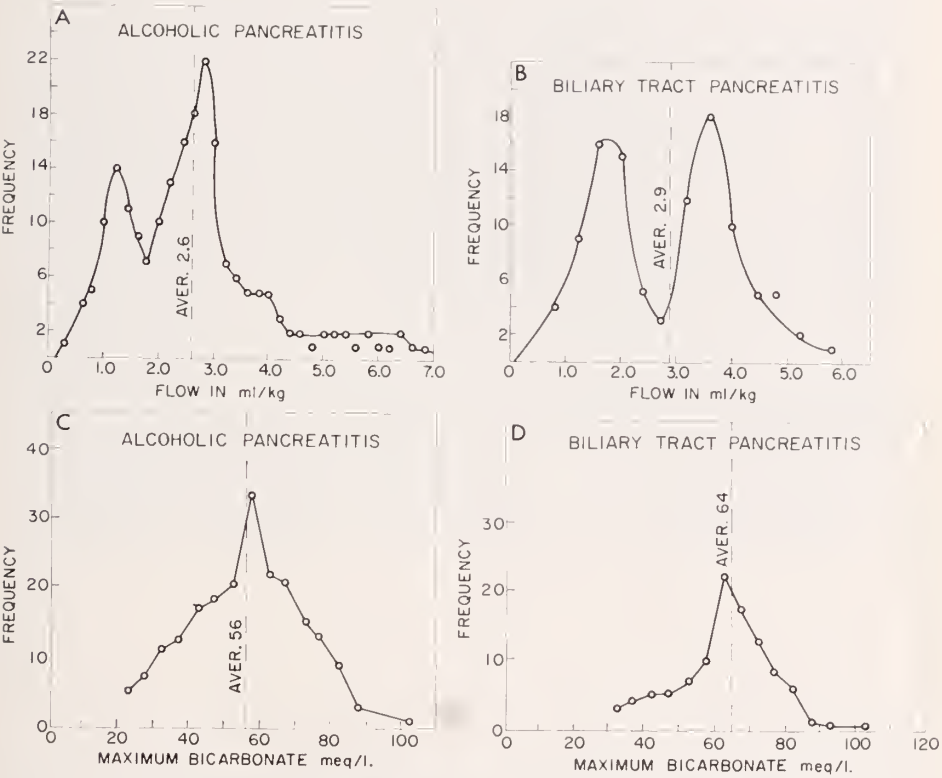

FIG. 1. Flow in alcoholic pancreatitis (A); flow in biliary tract pancreatitis (B); bicarbonate in alcoholic pancreatitis (C); bicarbonate in biliary tract pancreatitis (D).

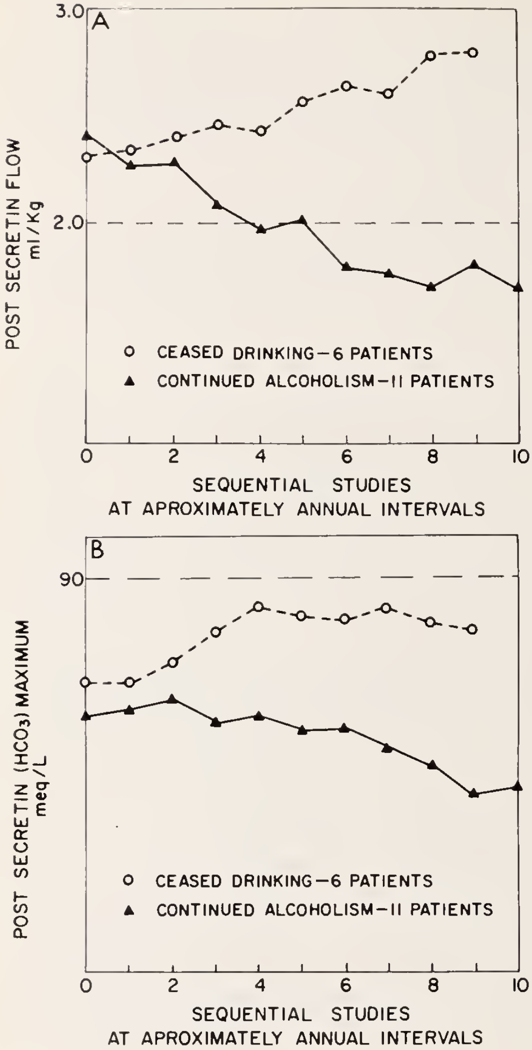

Fig. 2. Sequential annual secretin test data of alcoholics: (A) postsecretin flow and (B) bicarbonate.

Evidence for pancreatic regeneration and hypertrophy is abundant in the experimental animal and man. Additional support comes from sequential secretory data in alcoholics and histologic section of surgically removed pancreases. Figure 2 presents sequential annual secretin test data of 17 alcoholics. The data clearly show a decline in flow and a gradual decline in abnormally low maximum bicarbonate concentrations in the patients who continued to drink. On the other hand, in the patients who stopped drinking, the flow responses continued to rise and the maximum bicarbonate response increased and leveled off just below the lower limit of normal, 90 mEq/L. Thus, the pancreatic ductular cell mass can increase and decrease in response to injury and repair. Histologic section of pancreases removed from patients with acute recurrent or chronic relapsing pancreatitis corroborates the hypothesis of ductular regeneration, Figure 3.

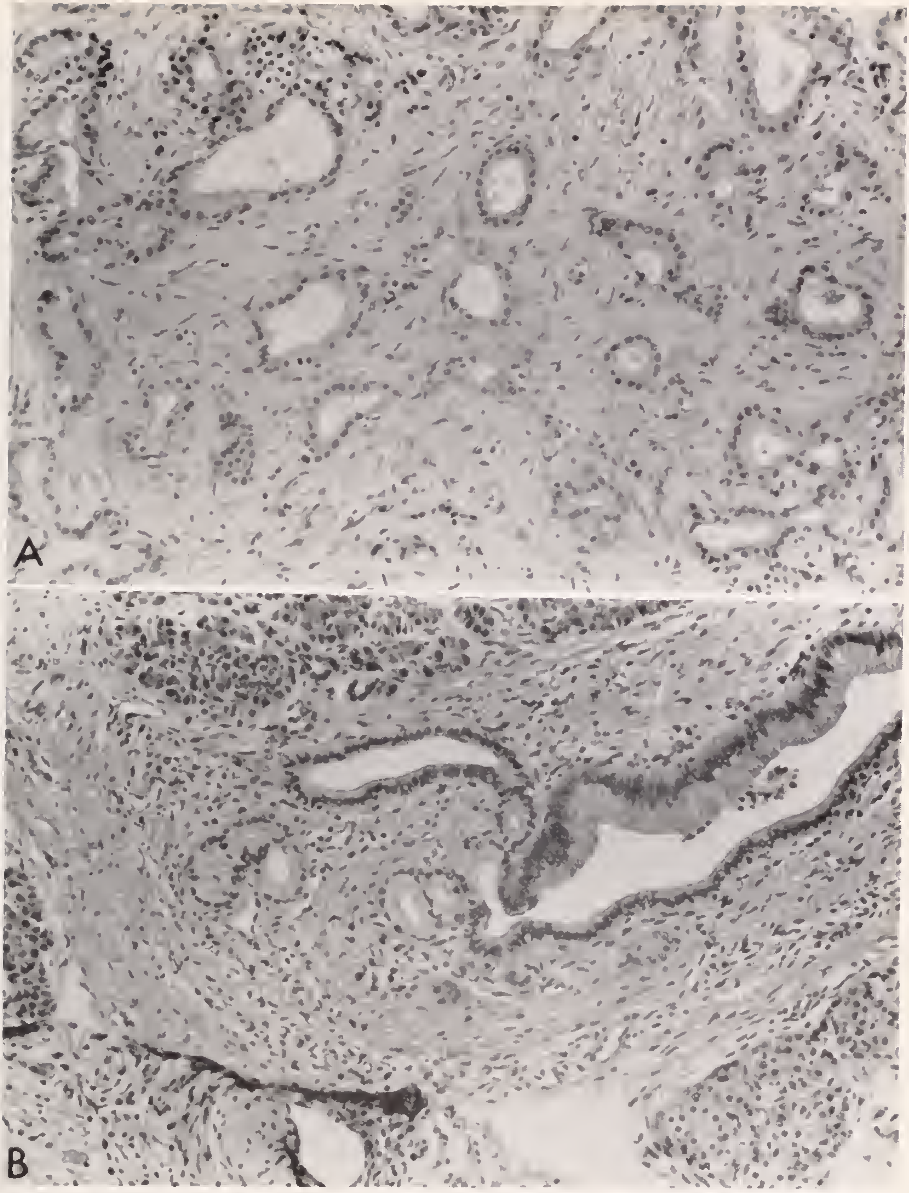

Fig. 3. Histologic section of pancreas—acute relapsing (A) and minimal chronic pancreatitis (B)—showing areas of new duct formation.

The concept (7) of an initial increase in pancreatic flow following pancreatic inflammation is in agreement with the recent reports of Sarles who describes an increased "proteinaceous" secretion in the ducts of rats made alcoholic and also with Spiro's view that the onset of clinical evidence of pancreatic inflammation is preceded by a long interval of minimal pancreatic inflammation.

## Summary and Conclusion

Analysis of the secretory data of patients with alcoholic and biliary tract chronic pancreatitis, of patients with alcoholism, and of patients with cirrhosis leads to the formulation of a new hypothesis which explains some of the confusion in the literature concerning the pattern of secretion in chronic pancreatitis and indicates, in addition, an inherent deficiency in the secretin test in the diagnosis of the most minimal lesions of chronic pancreatitis.

The findings suggest that the initial postsecretin secretory pattern of minimal pancreatic inflammatory pathology is hypersecretion, that is, increased flow, normal bicarbonate, normal enzyme, and a secretory pattern resulting from the initial reaction of the pancreas to injury, namely, ductular reduplication and hypertrophy. As the inflammation and fibrosis continues, the increased flows return toward normal statistical ranges, accompanied by a marked fall in bicarbonate concentration. The final stage of low flow, low bicarbonate, and low enzyme secretion corresponds, of course, to the more advanced pathologies of fibrosis, atrophy, and calcification.

Augmentation of ductular mass and consequent pancreatic secretory capacity occur in response to continued specific stimulation such as gastrin in Zollinger-Ellison or injury such as iron salt deposition in hemachromatosis.

Finally, the pancreatic ductular system, like the closely related hepatic ductular system, is not a network of passive conduits but actively participates in secretion and may be the dominant factor in determining the pattern of pancreatic secretion.

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# Cancer Mortality and Polyunsaturated Fatty Acids

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### Abstract

A report in the literature suggested "excess" mortality from cancer in subjects adhering to a diet high in polyunsaturated fatty acids while attempting to control the incidence of new coronary heart disease. Accordingly, the 13-year experience of the Diet and Heart Disease Study conducted by the New York City Health Department was examined from this aspect. Our observations lend no confirmation to the alleged association between excess cancer mortality and high PUFA diets.

Controlled clinical trials have established the effectiveness of a diet high in polyunsaturated fatty acids in lowering the serum cholesterol (1–4) and in reducing the significant effects of atherosclerosis (5–8). Nutritional safety of the diet has been demonstrated in terms of the adequacy of the serum levels of vitamins A and E (9) and $B_{12}$, C and carotene.* Pearce and Dayton (10) however, report that despite a significant reduction in fatal atherosclerotic events in the treatment group, the total mortality in treatment and control groups was virtually equal. The difference was largely made up by an excess of deaths due to cancer in the treatment group. The difference was of borderline statistical significance. This finding has raised once again the question of the safety of diets of this kind.

The Diet and Coronary Heart Disease Study of the New York City Department of Health in progress since 1957, tested the hypothesis that adherence to a serum-cholesterol-lowering diet is associated with decreased incidence of coronary heart disease. The design of the study has been described (1). Since its inception, 1764 men 40–59 years of age have come under long-term observation. Of this number, a control group of 533 has received the same initial work-up and reports to the study facilities for physical examination and blood chemistries, but is not offered dietary counseling. The remaining 1231 are in the experimental group for whom dietary changes were recommended and who may be further subdivided:

a) The *active* experimental group of 378 who attend the study facilities regularly for physical examination, nutrition counseling, and serum biochemical determinations according to a definite appointment schedule. A noninstitutionalized population, they do not necessarily adhere to the prescribed diet. However, serum cholesterol determinations and analyses of the fatty-acid com-

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* Unpublished data by G. Christakis, Nutrition Division, Department of Community Medicine, Mount Sinai School of Medicine of The City University of New York, N.Y. 10029.

TABLE I

*Number of Deaths by Study Group by Cause as of September 1970*

| Cause | Experimental | | Control |
|---|---|---|---|
| | Active | Inactive | |
| Heart disease* | 30 | 58 | 10 |
| Cancer | 6 | 15 | 10 |
| Other | 5 | 12 | 5 |
| Total | 42 | 85 | 25 |
| Person-years of observation | 5903 | 6156 | 4372 |

* Includes the group of subjects who entered the study with prior overt coronary heart disease. These subjects have never been included in prior reports since they could not add to the experience concerning new coronary heart disease. However, they have been included here since they can contribute to cancer experience.

TABLE II

*Cancer Mortality Rates per 100,000 by Group by Age at Entry, Total Study Experience since Start of Study June 1957*

| Group | Total | Age at Entry | |
|---|---|---|---|
| | | 40–49 | 50–59 |
| Experimental | | | |
| Active | 101.6 | 0 | 123.2 |
| Inactive | 243.6 | 0 | 358.7 |
| Total | 174.1 | 0 | 232.0 |
| Control | 228.7 | 87.4 | 383.8 |

position of their adipose tissue indicate that most in this group adhered to a high PUFA diet.

b) The *inactive* experimental group of 853, who were once active subjects but now limit their participation to appearance at the study facilities annually for physical examination or response to mail or phone inquiry to ascertain the status of study end-points. The two most important causes for change from active to inactive status have been lack of parking facilities near the study site and change in job situation whereby the subject could no longer devote the time necessary during regular working hours. Subjects in the inactive group did not necessarily divorce themselves from adherence to the study diet. Serum cholesterol determinations and fatty acid analyses indicate that many in this group still adhere to a high PUFA diet.

Gas liquid chromatographic analyses of the fatty acid composition of the subcutaneous adipose tissue indicated that the treatment and control groups have ingested diets significantly different in polyunsaturated fat content.*

The total mortality in the various groups during the 13 years of experience from 1957 to 1970 is seen in Table I.

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* Unpublished data by George Christakis, M.D.

In Table II, we see that the control group and the inactive treatment group had cancer mortality rates higher than that of the active experimental group. No attempt has been made to ascertain the significance of the differences between these rates since their validity could not be readily defended in a study in which coronary heart disease was the main interest.

Table III presents the deaths from cancer by primary site by study group. There is no significant aggregation by site.

Perspective may be gained by comparing observed mortality attributable to cancer in each group with expected deaths. Expected deaths were derived by application to 1969 cancer mortality rates for New York City white males in the appropriate age groups to the number of person-years of observation accumulated for each study group. For men who entered the study at 40–49 and

TABLE III

*Number of Deaths from Cancer by Study Group by Site, June 1957 to September 1970*

| Site | Experimental | | Control |
|---|---|---|---|
| | Active* | Inactive | |
| Esophagus | 1 | | |
| Stomach | | | 3 |
| Colon | 1 | 3 | 1 |
| Rectum | | 1 | |
| Pancreas | 1 | 1 | |
| Liver | | 1 | 1 |
| Larynx | | 1 | |
| Lung | 1 | 3 | 1 |
| Prostate | | 1 | |
| Spine | 1 | | |
| Brain | 1 | 1 | 1 |
| Lymphosarcoma | | 2 | 2 |
| Leukemia | | | 1 |
| General peritoneal | | 1 | |
| Total | 6 | 15 | 10 |

* Cases of cancer in inactive subjects developing within six months of becoming inactive have been charged to the active group.

TABLE IV

*Expected and Actual Number of Deaths from Cancer by Entry Age by Study Group*

| Group | 40–49 | | 50–59 | |
|---|---|---|---|---|
| | Observed | Expected | Observed | Expected |
| Experimental | | | | |
| Active | 0 | 1.6 | 6 | 23.3 |
| Inactive | 0 | 3.1 | 15 | 19.7 |
| Total | 0 | 4.7 | 21 | 43.0 |
| Control | 2 | 3.6 | 8 | 13.2 |

50–59 years of age, the New York City experience for men 45–49 and 55–59 years of age respectively was so used. These actual and expected numbers are compared in Table IV.

For each study group by age, the actual number of deaths from cancer was less than the expected number. Inasmuch as the hypothesis of excess cancer mortality was stated in the 1-tail form, the direction of the difference in experience between the groups shown in Table IV needs no statistical testing to indicate that the experience of the Diet and Coronary Heart Disease Study, through its first 13 years of operation, lends no confirmation to the suggestion of Pearce and Dayton.

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# Observations on the Relationship between Lymphosarcoma and Hodgkin's Disease

MARIAN WISNIEWSKI, M.D. AND CYRIL TOKER, M.D.

### Abstract

The continuity that exists between Hodgkin's disease and reticulum cell sarcoma is well recognized. The two cases presented illustrate a similar phenomenon at the opposite end of the lymphoma spectrum, namely, the merging of lymphocyte-predominant Hodgkin's disease with lymphosarcoma.

In the first case, the tumor, which initially exhibited a histologic appearance characteristic of lymphosarcoma, subsequently manifested a pleomorphic pattern indicating an evolution in the direction of Hodgkin's disease.

In the second case, patterns both of Hodgkin's disease and follicular lymphosarcoma were observed concomitantly in different lymph nodes.

The classification of the malignant lymphomas in general, and of Hodgkin's disease in particular, has been a subject of endless discussions and has undergone multiple corrections and modifications. The current division into lymphosarcoma, Hodgkin's disease, and reticulum cell sarcoma is a simple and a practical one, but unfortunately it frequently seems to please clinicians and statisticians more than it does pathologists. The boundaries between different malignant lymphomas are far from being clear-cut, and these tumors merge and transform into each other (1–7). There is an increased tendency to view Hodgkin's disease as a modification of lymphosarcoma or of reticulum cell sarcoma (2, 8). Pleomorphic reticulum cell sarcoma and Hodgkin's disease are considered by some authors to be closely related or of identical nature (2, 3, 8–11). The analogous relation of lymphosarcoma to Hodgkin's disease has not been equally stressed (2, 12).

The following report describes two cases of malignant lymphoma which demonstrate the intimate relationship between lymphosarcoma and Hodgkin's disease. Both cases illustrate the continuity that exists between lymphosarcoma and lymphocyte-predominant Hodgkin's disease.

### Case Reports

*Case 1.* A 58-year-old woman was admitted to the hospital with a flank mass, which allegedly had been present for ten years and had increased in size during the past year. Physical examination revealed a nontender, firm, circumscribed, movable, subcutaneous mass about four centimeters in size, located in the right loin area. There were no palpable lymph nodes and no organomegaly. The chest x-ray revealed no abnormality. Excision of the mass was performed.

*Pathologic examination:* Grossly, the mass consisted of a soft elastic, tan node measuring 5 × 2.5 × 2.5 cm. Microscopically, the tumor was composed predominantly of lymphocytic cells. Monotonous sheets of small and medium-size lymphocytes were seen, the malignant

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cells infiltrating the subcutaneous tissues (Figs. 1 and 2). There was no tendency toward follicle or nodule formation. The diagnosis was lymphosarcoma.

Following the operation local radiotherapy was given. The patient remained well for 12 years, at which time she was again admitted and examination revealed axillary mass, which was examined by biopsy.

*Pathologic examination:* The pleomorphic histologic appearance contrasted noticeably with the monotonous composition of the initial biopsy. The neoplastic proliferation was composed now of lymphocytes with a significant admixture of reticulum cells (Fig. 3). The lymphocytes were of medium size. Cells similar to so-called "lacunar cells" were observed (Fig. 3). Bi- and multinucleated reticulum cells similar to Reed-Sternberg cells were seen (Fig. 4). They lacked, however, the large nucleoli characteristic of the typical Reed-Sternberg cells. Polymorphonuclear neutrophilic and eosinophilic leukocytes were interspersed among other cells and produced occasional microabscesses (Fig. 5). Fibrosis was not a prominent feature. The diagnosis was Hodgkin's disease.

At the time of the second operation and thereafter the patient's chest x-rays were normal and the liver and the spleen were not palpated. Fifteen years following the first operation the patient remained well, her only complaint being an enlarged left (opposite) axillary node, for which she was treated with radiotherapy.

*Case 2.* A 32-year-old man was admitted to the hospital with a left cervical mass of unknown duration. No other lymph nodes were palpated. The liver and the spleen were not enlarged, and the chest x-ray examination was within normal limits. Excision of the cervical node was performed.

*Pathologic examination:* The normal architecture of the lymph node was destroyed and sheets of uniform lymphocytes predominated. In places, the lymphocytes were arranged in a follicular pattern similar to follicular lymphosarcoma (Fig. 6). However, in other areas the admixture of reticulum cells and classical Reed-Sternberg cells led to the diagnosis of Hodgkin's disease.

Four weeks later a laparotomy and splenectomy were performed. Specimens were taken from the liver and lymph nodes for biopsy.

*Pathologic examination:* The spleen as well as multiple lymph nodes were involved with malignant lymphoma; the liver samples were free of the disease. Noteworthy differences in the histologic patterns were observed among individual involved lymph nodes.

Within some nodes the morphologic appearance was that of a follicular lymphosarcoma. In these areas the tumor was composed entirely of medium-size lymphocytes arranged in a nodular pattern (Fig. 7). The follicles coalesced in many places. Infiltration of the perinodal fatty tissue was noted. Reed-Sternberg cells or large reticulum cells were not present.

However, obvious Hodgkin's disease was seen in other locations. In some regions connective tissue septa produced a nodular pattern (Fig. 8). Lymphocytes constituted the predominant neoplastic cell type. Dispersed among them were various numbers of histiocytes, reticulum cells, and Reed-Sternberg cells (Fig. 9).

The patient was discharged for radiotherapy.

## Discussion

Our understanding of malignant lymphoreticular tumors has progressed to the realization that rigid separation of certain lymphomas may be artificial. Transitions have been reported between the different forms of Hodgkin's disease (13) and between various malignant lymphomas (1–7). An intimate relationship of reticulum cell sarcoma and Hodgkin's disease is accepted by some (2, 8). It would appear probable that at the opposite end of the lymphoma spectrum lymphosarcoma merges with Hodgkin's disease of lymphocytic type. The two cases presented document this phenomenon.

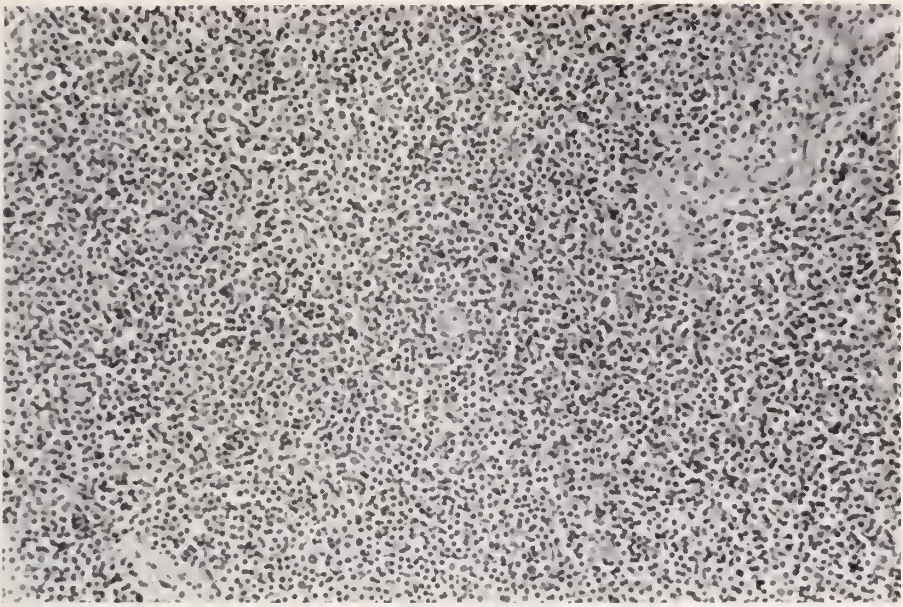

Fig. 1. Case 1: Low power view of the first biopsy illustrating malignant lymphoma composed of homogeneous sheets of small and medium-size lymphocytes (H&E × 100).

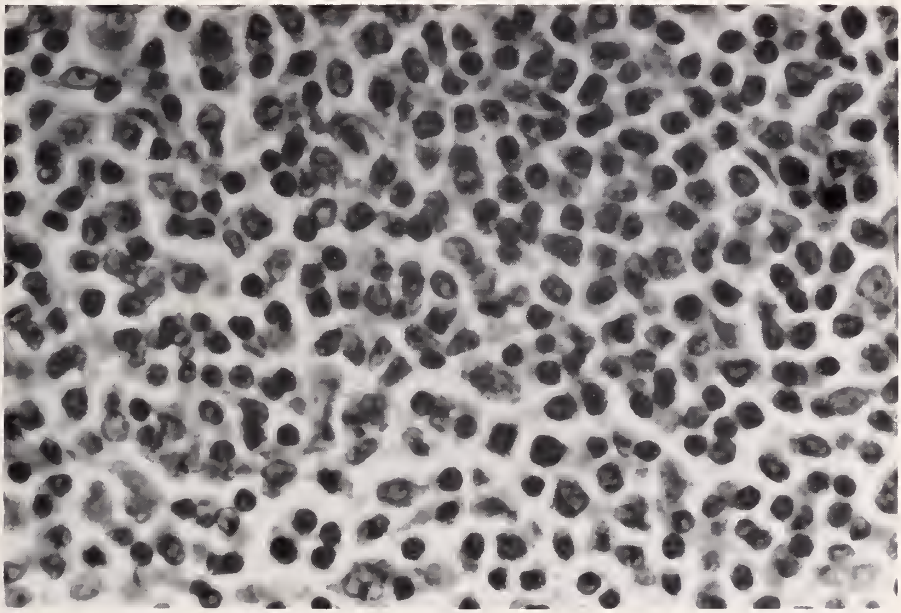

Fig. 2. Case 1: Higher power of Fig. 1. A homogenous lymphocytic infiltrate is shown (H&E × 400).

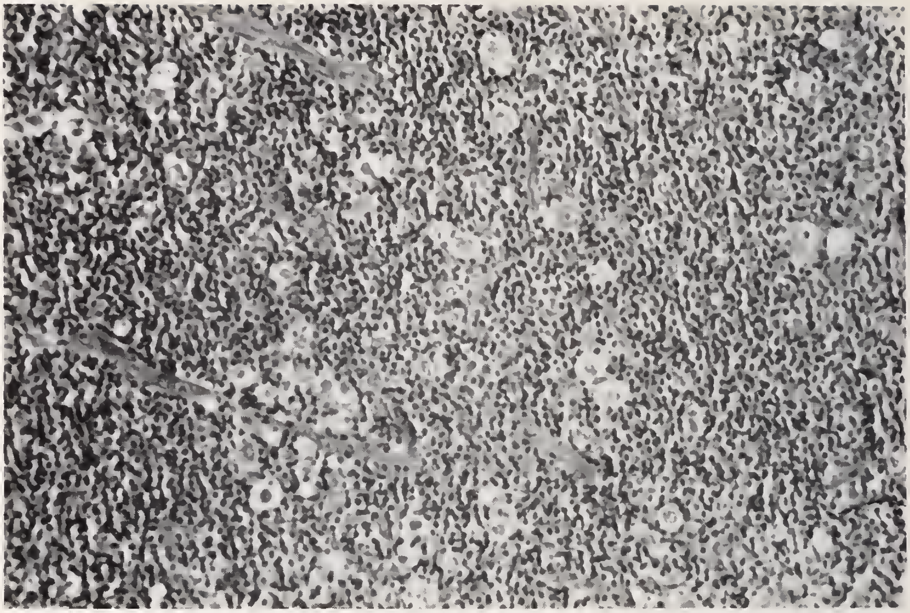

Fig. 3. Case 1: Second biopsy. The lesion is pleomorphic and reticulum cells are conspicuous (H&E × 100).

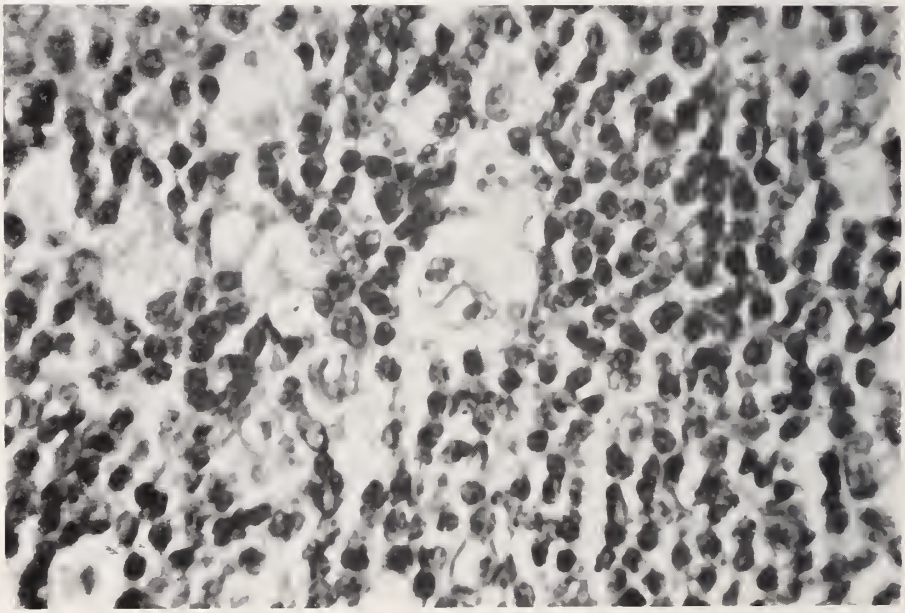

Fig. 4. Case 1: Second biopsy. A large binucleated reticulum cell is shown (H&E × 400).

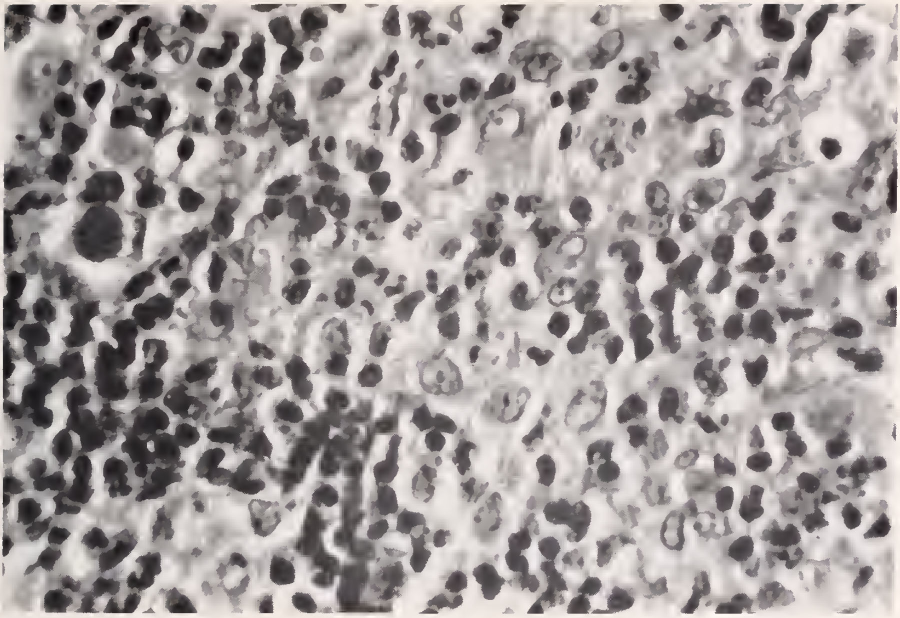

Fig. 5. Case 1: Second biopsy. Cellular pleomorphism is pronounced. Lymphocytes, polymorphonuclear leukocytes and reticulum cells are shown H&E × 400).

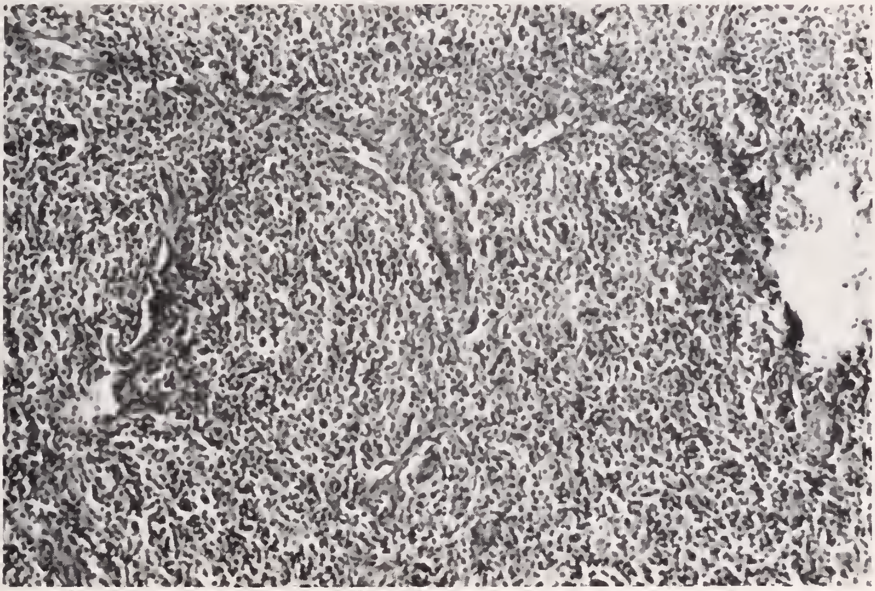

Fig. 6. Case 2: Cervical node. Follicles composed of lymphocytes are shown (H&E × 100).

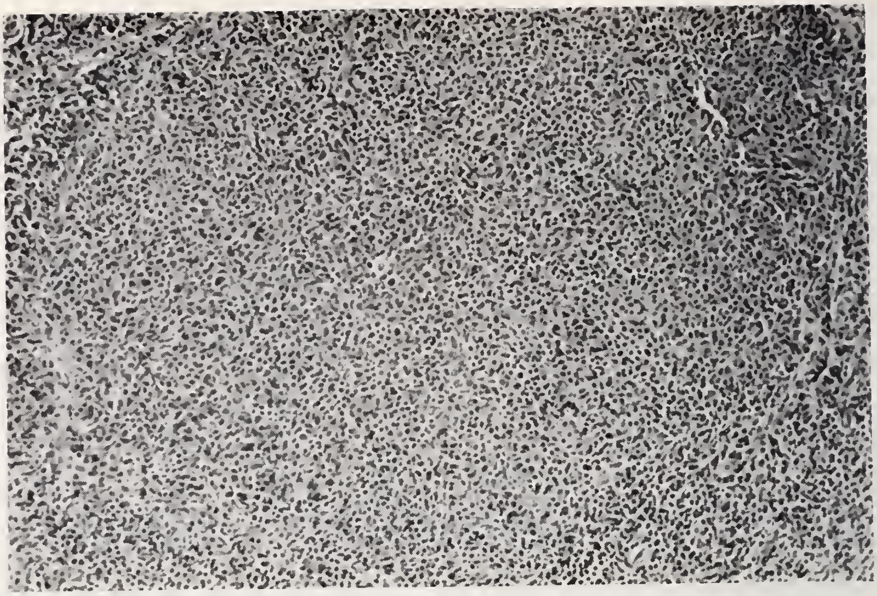

Fig. 7. Case 2: Abdominal node. The pattern is that of follicular lymphosarcoma (H&E × 80).

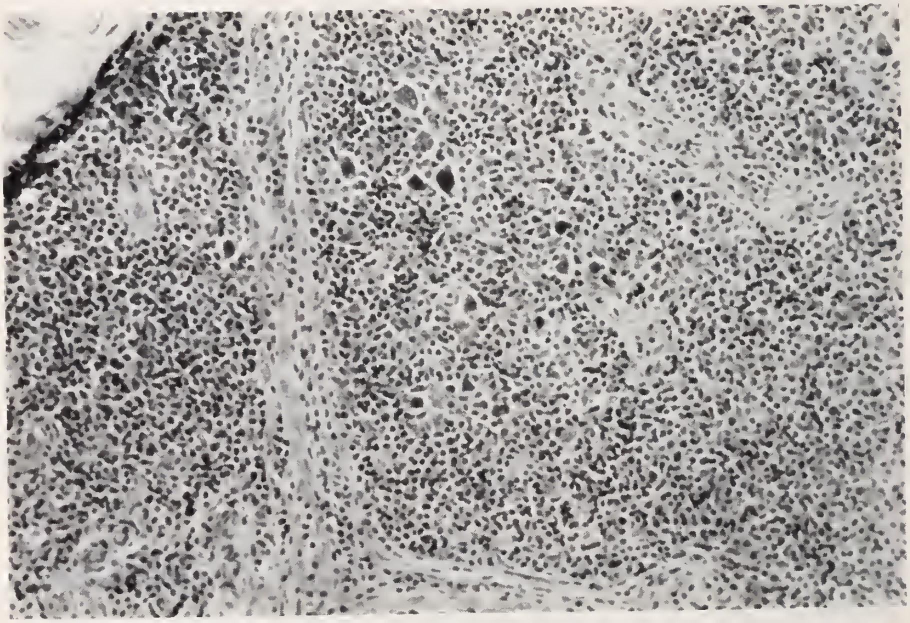

Fig. 8. Case 2: Abdominal node. A follicular pattern is evident (H&E- × 100).

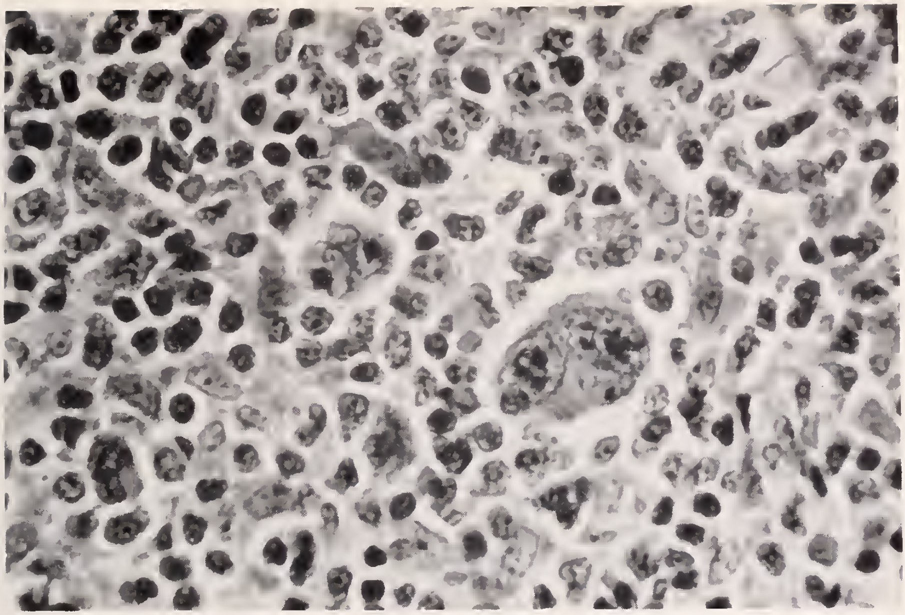

FIG. 9. Case 2: Abdominal node. Typical Reed-Sternberg cells are shown (H&E × 400).

In the first case, the original diagnosis was diffuse lymphosarcoma. After an unusually long period of twelve years another mass appeared, which could not be similarly designated. Instead, the histologic features indicated a trend in the direction of Hodgkin's disease. Cellular pleomorphism denoted the development of Hodgkin's characteristics, despite the absence of classical Reed-Sternberg cells.

In the second case, a characteristic lymphosarcoma underwent transformation to Hodgkin's disease with lymphocytic predominance, with numerous classical Reed-Sternberg cells and cellular pleomorphism.

Hodgkin's disease may occupy an intermediate position in the spectrum of the malignant lymphomas, being located between lymphosarcoma at one end and reticulum cell sarcoma at the opposite end. At its two extremes Hodgkin's disease merges into the other lymphomas. In some cases precise classification is impossible. Whereas the confluence of Hodgkin's disease and reticulum cell sarcoma is well recognized, the continuity between lymphosarcoma and lymphocyte-predominant Hodgkin's disease may deserve equivalent emphasis.

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# The Coexistence of An Ovarian Brenner Tumor and Endometrial Stromal Sarcoma

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## Abstract

Endometrial stromal malignancy has been noted to occur in association with hormonally active theca cell tumors of the ovary. The present report documents the coexistence of an ovarian Brenner tumor and an endometrial stromal sarcoma. The hormonal capability of the ovarian neoplasm was manifested by pronounced endometrial glandular hyperplasia. The possibility that stromal neoplasia may be induced by estrogen stimulation is discussed.

## Introduction

The association of Brenner tumors of the ovary with malignant transformation of the endometrial glands is a rare but well-known phenomenon (1–3). These observations have been interpreted as evidence for the secretion of estrogen by the stromal component of this ovarian neoplasm. Recently the coexistence of theca cell tumors and endometrial stromal malignancy has been described (4). The present brief report concerns the association of an ovarian Brenner tumor with an endometrial stromal sarcoma.

## Case Report

A 49-year-old woman was operated on for vaginal bleeding of four weeks' duration. A preliminary curettage revealed hyperplastic endometrial glands and a stroma that was considered to be sarcomatous. The hysterectomy specimen revealed a fleshy, polypoid, yellow tumor that extended deeply into the myometrium and measured 9.5 × 6.5 × 5.0 cm (Fig. 1). Histologic examination of the neoplasm revealed tightly spaced sheets of spindle cells with hyperchromatic nuclei infiltrating the myometrial tissue (Fig. 2). The endometrial glands both adjacent to and remote from the stromal tumor showed cystic glandular hyperplasia, but no evidence of carcinoma (Fig. 3). The left ovary contained a well-circumscribed tan neoplasm measuring 1.5 cm in diameter which proved on histologic examination to be a Brenner tumor (Fig. 4).

## Discussion

The secretion of estrogen by theca cell tumors is well recognized, as are the associated findings of endometrial hyperplasia and carcinoma (5). Of rarer occurrence is the association of Brenner tumors with malignant transformation of the endometrial glands (1–3).

The stromal component of the Brenner tumor appears, in some instances, to be capable of producing estrogenic substances (2). Substantiation for this hypothesis lies in the finding of fat within the stromal cells of the tumor as well as in its previously mentioned association with endometrial hyperplasia and carcinoma (2). The sensitivity of endometrial glands to estrogenic stimulation is apparent. The proliferative response of the endometrial stroma to estrogenic

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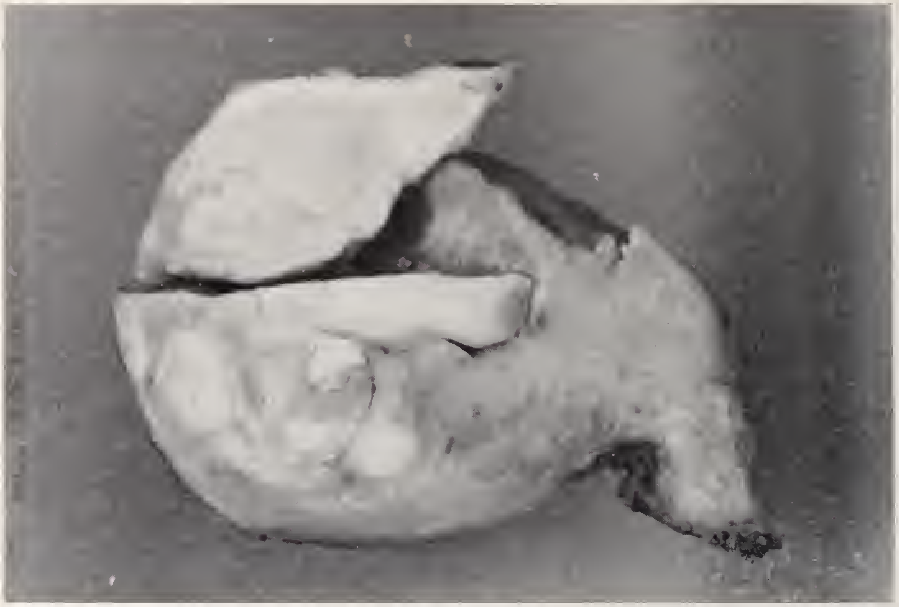

FIG. 1. Uterus showing polypoidal tumor extending deeply into the myometrium.

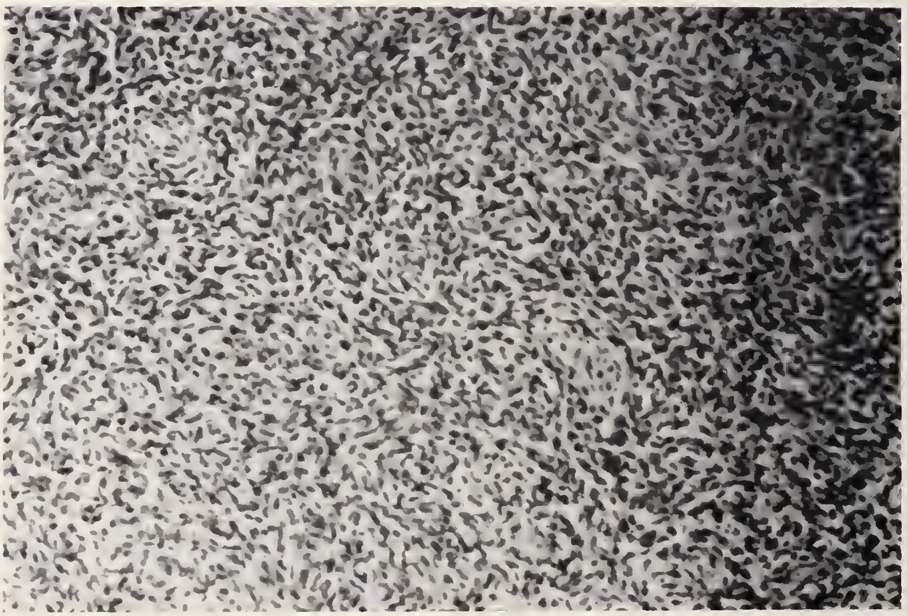

FIG. 2. Photomicrograph showing compact sheets of spindle-shaped tumor cells (hematoxylin-eosin × 100).

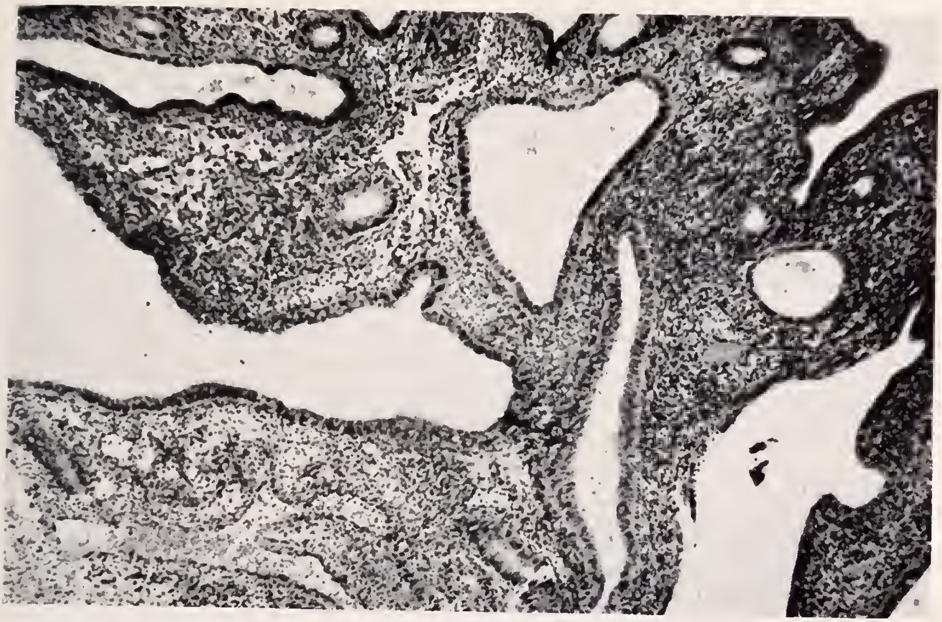

FIG. 3. Photomicrograph of endometrium showing cystic glandular hyperplasia (hematoxylin-eosin × 40).

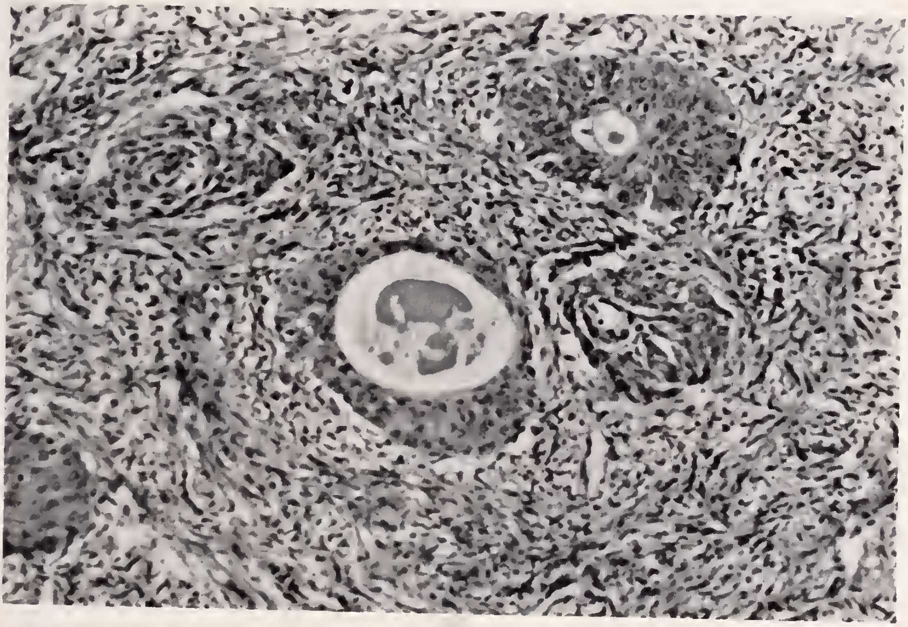

FIG. 4. Photomicrograph showing ovarian Brenner tumor (hematoxylin-eosin × 100).

stimulation should not, however, be overlooked. This is best exemplified by its activity in the proliferative phase of the menstrual cycle. Toker recently described two cases in which an endometrial stromal malignancy coexisted with a theca cell tumor and raised the possibility of their being causally related (4). The present report documents the coexistence of an endometrial stromal sarcoma and an ovarian Brenner tumor. In addition, the endometrial acini showed cystic glandular hyperplasia. This last finding, in conjunction with previous reports, suggests that the ovarian neoplasm was secreting estrogenic substances capable of stimulating the endometrial glands. A causal relationship similarly mediated between the ovarian tumor and the stromal sarcoma immediately presents itself, particularly in view of our previous experience (4). Further reports of similar occurrences, particularly if accompanied by assays of the ovarian tumor for estrogen, may well substantiate the belief that hormonally active ovarian tumors are capable of inducing endometrial stromal neoplasia.

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# NEUROLOGIC NOTES

## Simultaneous Bilateral Focal Seizures without Loss of Consciousness

JESSE WEINBERGER, M.D. AND JOHN LUSINS, M.D.

Focal seizures are a common manifestation of some cerebral lesions, particularly neoplasms. Unilateral seizures often occur without disturbance of consciousness; however, it is thought that when seizure activity crosses the midline, there uniformly is a loss of consciousness. (1) The following is a case report of a 64-year-old woman shown to have a parasaggital meningioma who exhibited seizures without loss of consciousness involving midline axial structures and both upper extremities.

### Case Report

E.S. is a 64-year-old woman who stated that for the previous twelve years she had frequent brief spontaneous movements of the tongue, lip-smacking, and elevation of the eyebrows with wrinkling of the forehead. For two years prior to admission she also had episodes during which she could not speak and had uncontrolled movements of both hands. She had eight such episodes, each lasting twenty to thirty seconds. During these episodes she was aware of what was happening to her and could understand what people were saying, but when she tried to respond, she could only utter a phrase such as "uh-uh-uh." Her head and neck turned to the right. Both arms moved to the right and turned rhythmically on their axis at the wrist in a fashion best described as turning two door knobs, but in opposite directions. Sometimes the forearm would be involved in the rotation.

On neurologic examination the patient was alert and orientated. Speech was fluent and comprehension was good. However, she had some difficulty in use of language, such as reciting the alphabet, sentence repetition, and rhyming. She also had some difficulty in calculating. The cranial nerves were intact, including visual fields and pupillary responses. However, she had intermittent rapid movements of the tongue and eyebrows. There was no pallatal myoclonus. Gait and power were normal, the reflexes were symmetrical. Sensory examination was normal. Lumbar puncture showed on opening pressure 170 with clear fluid, no cells and a protein of 40 mg percent. The electroencephalogram showed diffuse slowing, greater on the left than the right. The brain scan showed a large, well-delineated area of uptake in the parasagittal region (Figs. 1 and 2). Angiography showed a large vascular mass, located parasagittally, more on the left, but crossing the midline (Figs. 3 and 4), and compatible with a meningioma.

### Discussion

The involuntary movements of the tongue, lips, and brow were suggestive of a seizure focus in the midline, possibly in the brain stem. Nathanson has reported three cases with intermittent, involuntary movements of midline axial structures such as the mouth, tongue, and palate, which he interpreted as a form of seizure discharge. The lesions were in the posterior fossa. (2)

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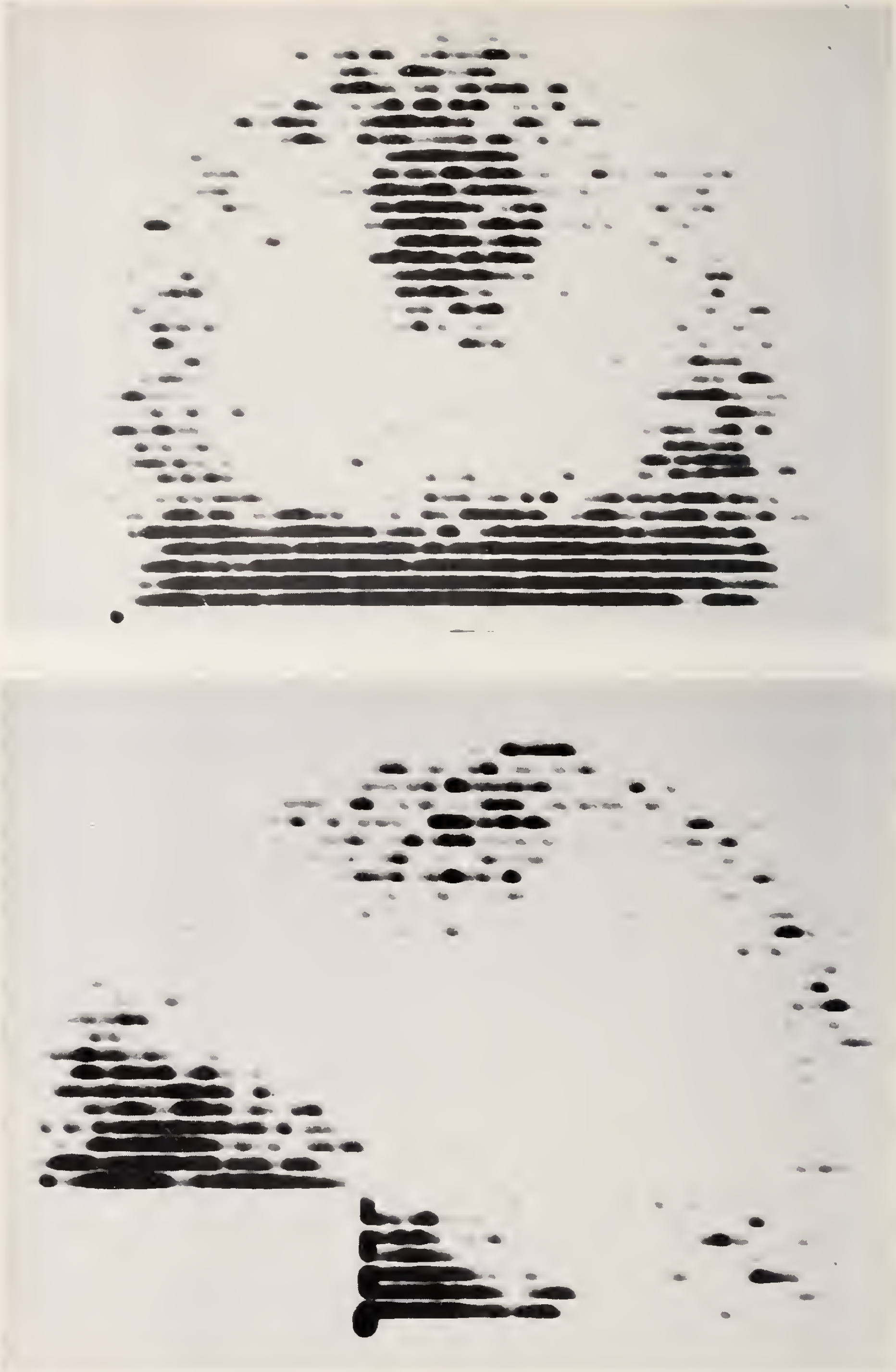
The brain scan was provided by Dr. M. Budabin of the Scan Laboratory (New York City).



FIGS. 1 and 2. Technetium 99m brain scan—anterior and lateral view—showing a large midline mass compatible with a meningioma.

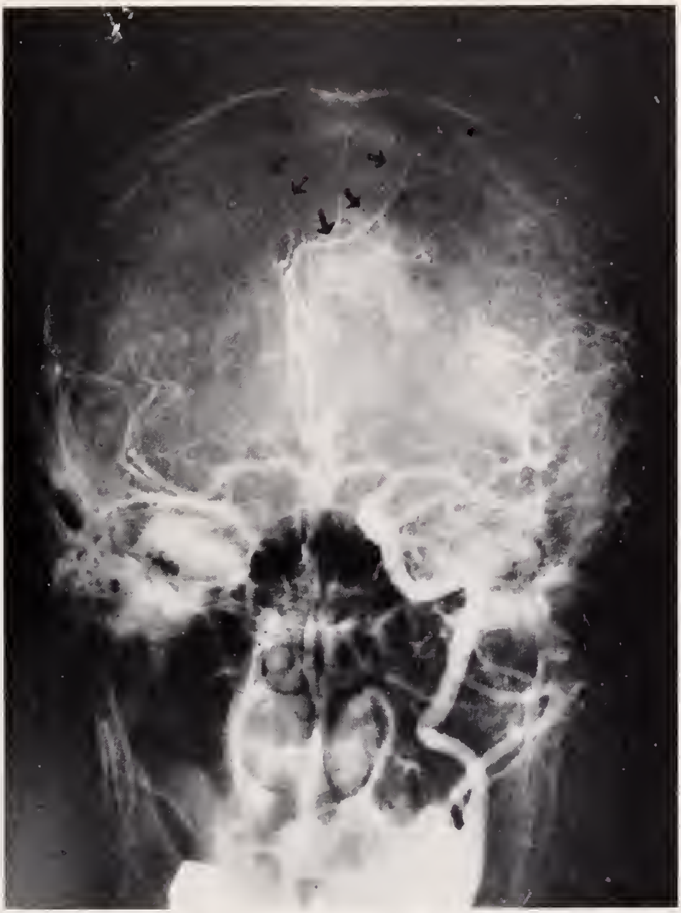


FIG. 3. Anterior view of cerebral angiogram arterial phase showing lateral and downward displacement both of right and left anterior cerebral arteries (arrow).

The movements of both upper extremities and difficulties with speech could be interpreted as being of a temporal lobe origin, since the movements and the speech had a stereotyped repetitive nature. This type of activity has often been described in psychomotor seizures. However, in psychomotor seizures there is usually disturbance of consciousness (3) and this was never documented in our patient.

Bilateral weakness, particularly in the lower extremities is at times noted in parasagittal meningiomas. This would be expected since meningiomas in this location frequently compress both cerebral hemispheres. Bilateral seizure activity without loss of consciousness, on the other hand, appears to be a distinctly rare type of presentation. Gaufier-Smith reviewed 222 cases of parasagittal meningiomas. (4) Forty-nine percent of these patients presented with seizures, 27 percent were focal and did not lose consciousness. None of these individuals had simultaneous seizures on the two sides of the midline.

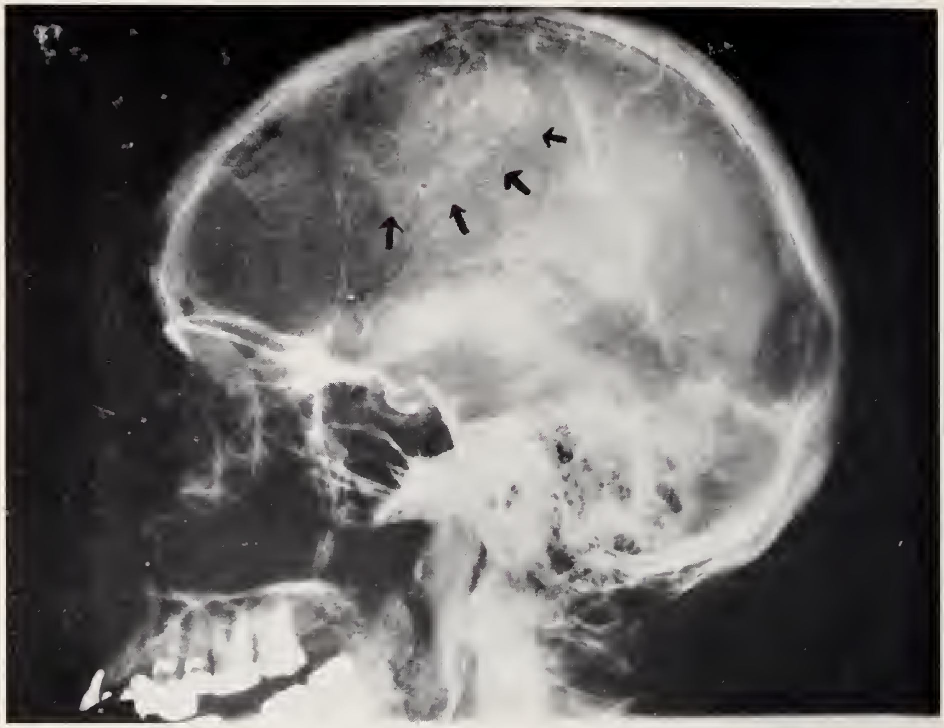


FIG. 4. Lateral view of cerebral angiogram, venous phase, showing an anteriorly located mass, as outlined by arrows.

## Summary

We have presented a patient with a parasagittal meningioma with a twelve-year history of uncontrolled movements suggestive of seizures. These involved axial structures and both extremities without the loss of consciousness, a rare and interesting presentation.

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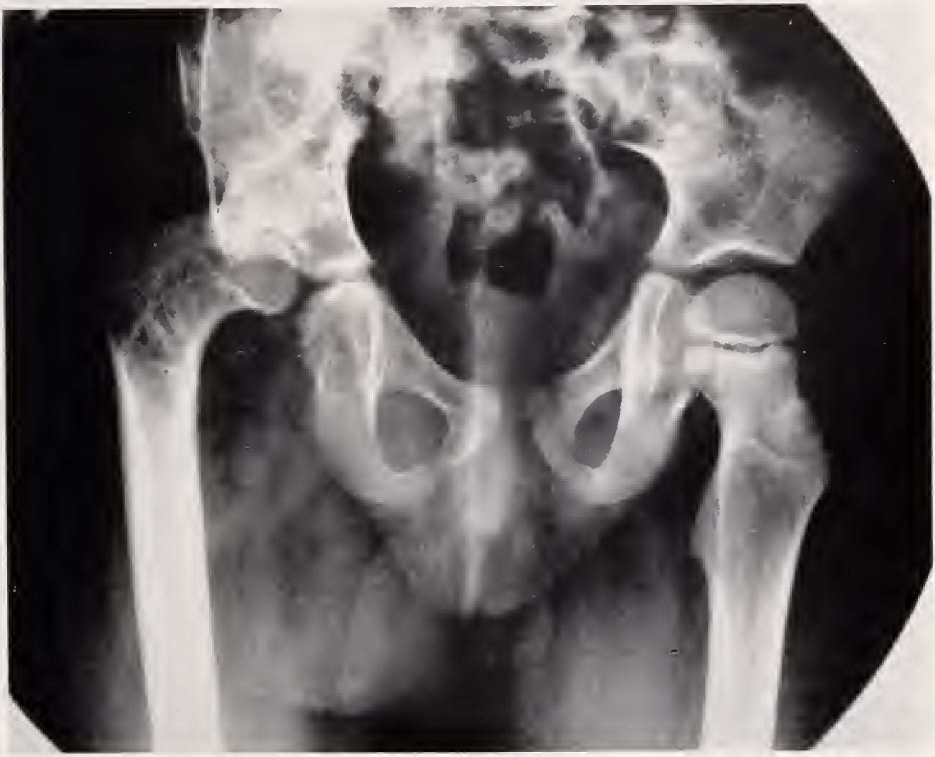
# RADIOLOGIC NOTES

CLAUDE BLOCH, M.D. AND HARVEY M. PECK, M.D., COEDITORS

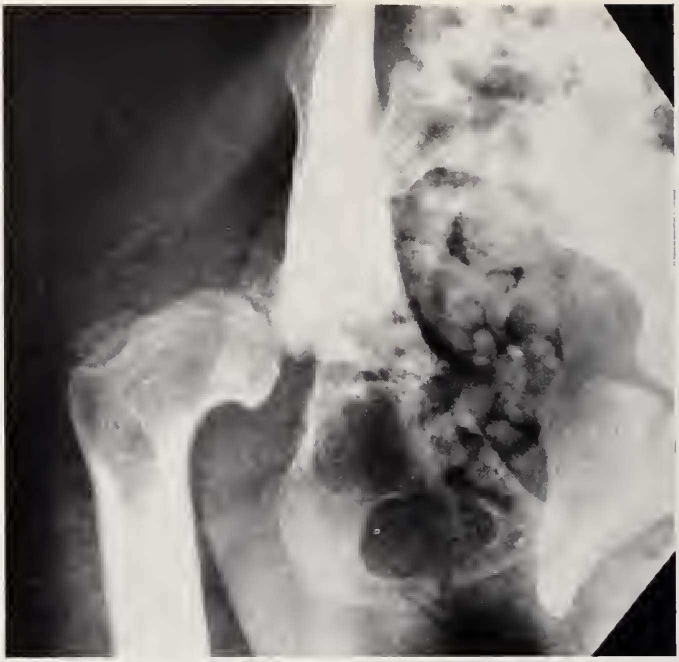
## CASE NO. 364

A four-year-old girl was known to have congenital dislocation of the right hip since birth. Conservative therapy had been unsuccessful in correcting the dislocation and four surgical procedures had also been performed without permanent improvement. The patient was admitted for adductor tenotomy and skeletal traction with a formal arthroplasty planned for the future.

Radiographic study of the pelvis and hips was performed. In the frontal projection, the right femoral head and neck were dislocated superiorly and presumably posteriorly; the femoral neck was broadened and deformed and the acetabular margins were irregular (Fig. 1). In the left posterior oblique view,



Case 364, Fig. 1. Antero-posterior view of the pelvis and hips shows superior and presumably posterior dislocation of the right femoral head. There is broadening and deformity of the head and neck and some irregularity of the acetabulum on the right. The left side shows coxa valga but no intrinsic abnormality.

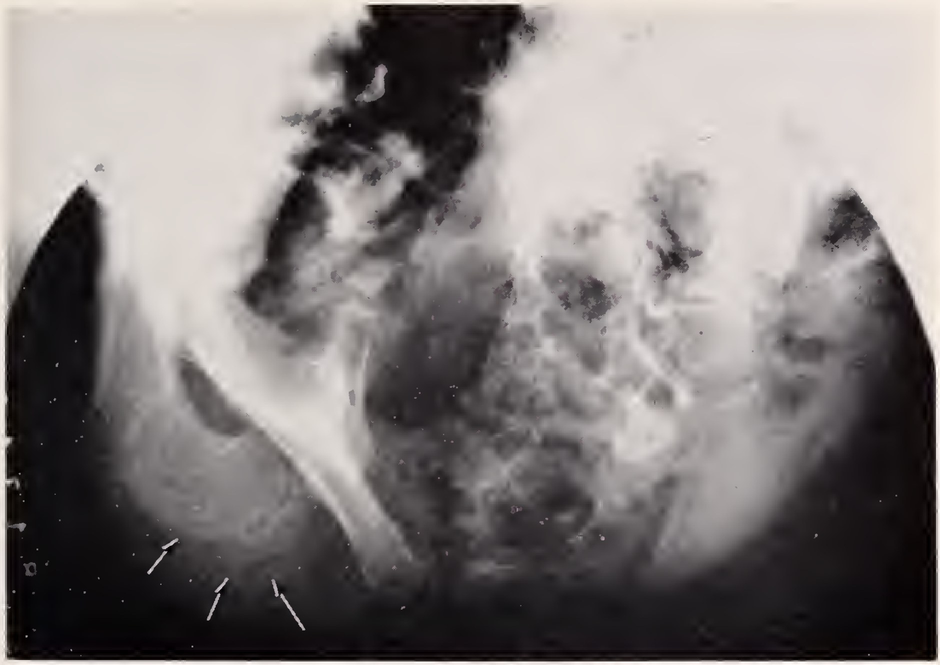


Case 364, Fig. 2. Left posterior oblique projection demonstrates a deformed right capital femoral epiphysis. The epiphysis is no longer superimposed on the acetabulum as in Figure 1, thus establishing its posterior location.

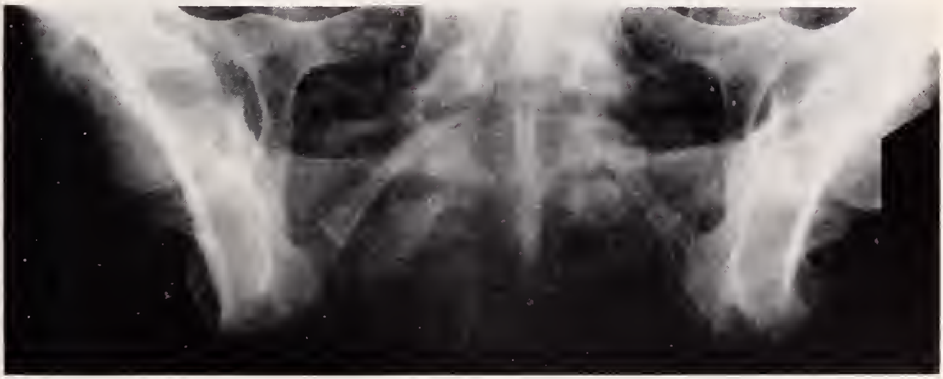
the deformed capital femoral epiphysis could be clearly seen and its position in this projection confirmed that the dislocation was posterior (Fig. 2). In addition to conventional projections and stereoscopic views, a Chassard-Lapine view was also performed (Fig. 3). The location of the right femoral head in a markedly posterior position was strikingly demonstrated, even more so when compared with the normally located left hip. A normal chassard-Lapine view is reproduced for comparsion (Fig. 4).

## Discussion

The Chassard-Lapine view is an axial view of the pelvis. It is produced by having the patient bend forward in full flexion at the hips with the central ray then entering vertically approximately through the lumbo-sacral joint. This projection has been most often employed in barium enema examinations where it is useful in clarifying the overlapped sigmoid and recto-sigmoid zones by affording a right-angled view of these structures compared with the conventional projections. Its value in elucidating orthopedic problems about the hip joints is less well known and this case points up this usefulness. Even though oblique and stereoscopic views will generally suffice in making the diagnosis of a dislocation, additional perspective is gained from the Chassard-Lapine projection. If the patient is unable to cooperate in positioning, the central ray may be angled



Case 364, Fig. 3. Chassard-Lapine view of the pelvis produced with the patient flexed completely at the hips and the central ray entering vertically through the lumbo-sacral joint. The right femoral head and neck are quite posterior in location behind the acetabulum (Arrows); compare this position with the normal position on the opposite side.



Case 364, Fig. 4. A normal Chassard-Lapine view is reproduced here for comparison.

cephalad with the patient supine or caudad with the patient prone to yield similar but not identical information; again, barium enema examination is the usual application of these angled projections.

*Case Report:* CONGENITAL DISLOCATION OF THE HIP DEMONSTRATED BY CHASSARD-LAPINE PROJECTION.

### Acknowledgment

This case was presented through the courtesy of the late Dr. Joel B. Alder, Good Samaritan Hospital, Suffern, New York.

## CASE NO. 365

A nine-year-old boy was brought to the doctor's office and then admitted to the hospital because of cough, abdominal pain, and fainting. He had had bronchitis and had been pale, tired, and fatigued for a few days prior. He fainted a number of times during his physical examination. Hemoglobin was 7.4 grams. A low enema returned a black, tarry stool.

Chest x-ray was normal. GI series revealed a large 5 cm ulcerated mass on the posterior wall of the fundus of the stomach (Figs. 1 to 3). The mass was round with smooth, sharp margins. A large, deep, irregular central "collar button" type ulceration was present which measured 2.5 cm in diameter. The diagnosis of an ulcerated submucosal tumor such as leiomyoma was suggested.

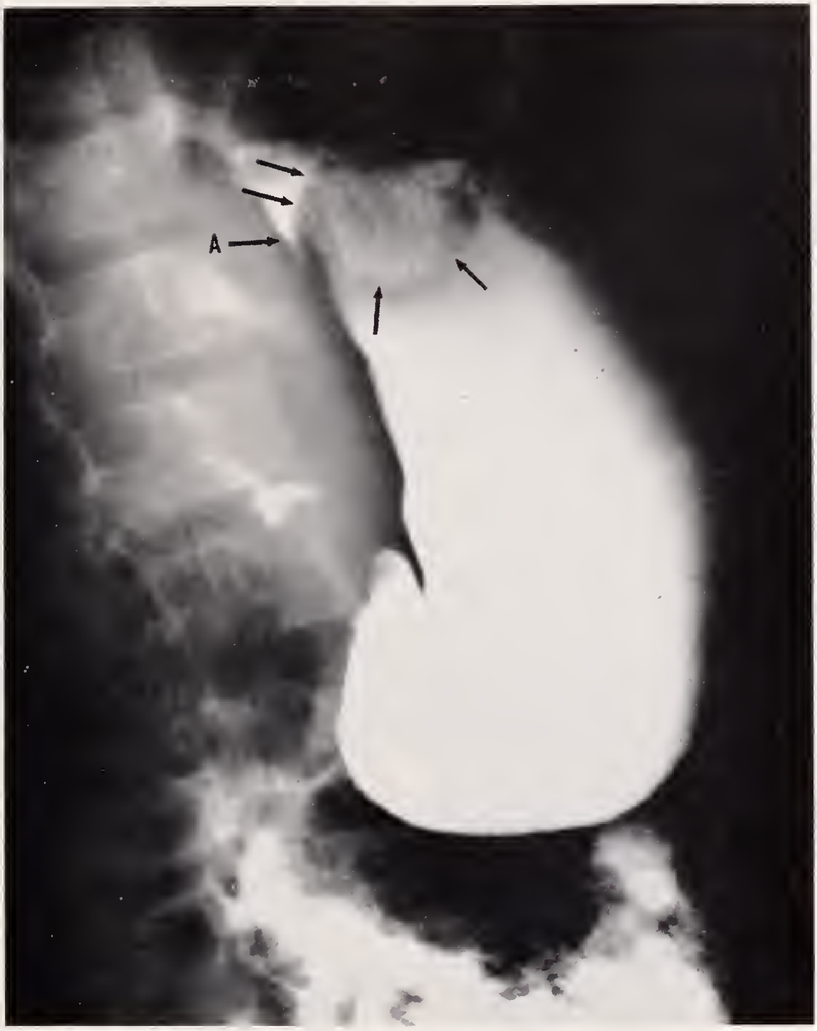
Local excision of the tumor mass was performed. Postoperative course was unremarkable and the patient is well one year after surgery. The pathologist reported an ulcerated benign leiomyoma of the stomach with mucosal alterations (bizarre smooth-muscle cell tumor of Stout).

### Discussion

Smooth-muscle tumors of the GI tract are common in adults but quite rare in children. The pathologist has difficulty in differentiating the benign leiomyoma from the malignant leiomyosarcoma and experience has shown that the most reliable histologic criterion is the number of mitotic figures observed. As in the adult, the ultimate confirmation of the degree of benignity remains long-term patient survival. While it is generally assumed that sarcoma develops in a preexisting benign tumor, there is apparently no direct evidence to substantiate this concept.

The roentgenographic features should suggest the correct diagnosis of submucosal tumor: a round mass related to the gastric wall with smooth, very sharp margins indicative of overlying stretched but intact mucosa (Figs. 1 and 2). The excavating central "collar button" ulceration is typical (Fig. 3), but even when irregular does not permit a differentiation between benign and malignant tumors.

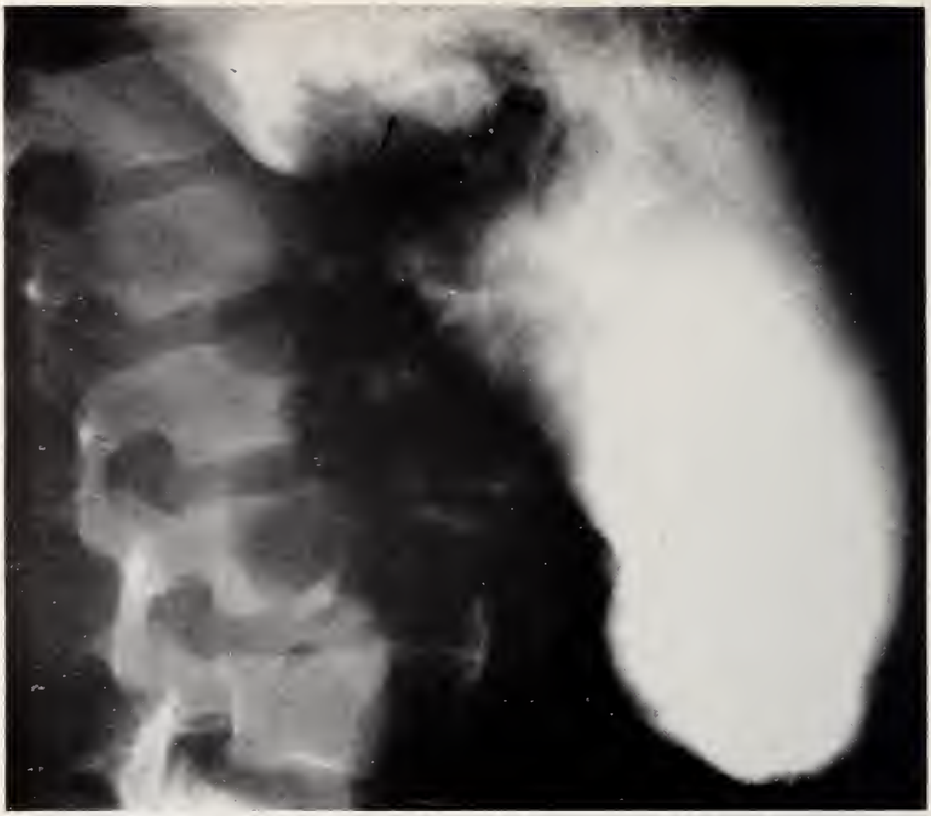
One confusing point is noted in the analysis to determine the site of origin of the mass, that is, mucosal versus submucosal. In Figure 1, the mass appears to make an acute angle with the posterior gastric wall (Arrow A). Such an acute



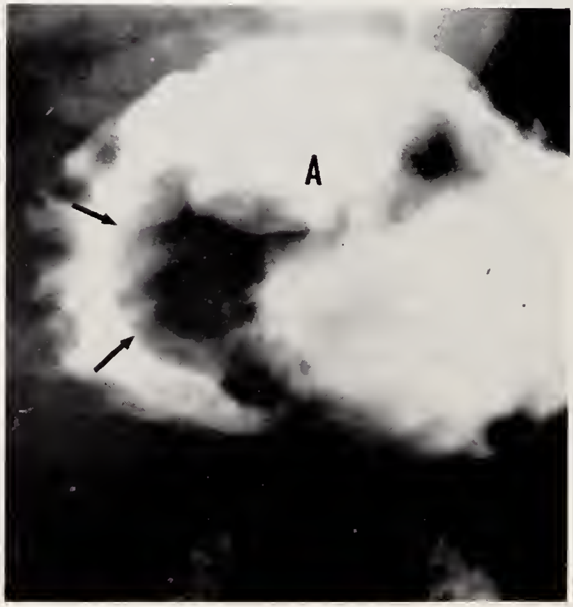
Case 365, Fig. 1. Oblique erect view of the barium filled stomach from GI series shows a 5 cm lucent filling defect located high on the posterior wall just below the air-barium fluid level. The margins of the lesion are exquisitely sharp (upper arrows). A central 2.5 cm rounded ulceration is present (lower arrows). The lesion appears to make an acute angle with the gastric wall (arrow A). Note, however, that the mass is viewed en face while the gastric wall is in profile; see text for significance.

angle is usually a reliable sign of a mucosal origin; however, the validity of this sign depends on a profile view. Note that although the mass is being viewed en face, the posterior gastric wall is in profile. The acute angle is thus more apparent than real and an incorrect conclusion of a mucosal origin of the mass is to be avoided.

*Case Report:* GASTRIC LEIOMYOMA IN A CHILD.



Case 365, Fig. 2. Lateral view again shows the large lesion with central ulceration (arrows).



Case 365, Fig. 3. Spot radiograph (patient supine, partially turned to right posterior oblique position) again shows exquisitely smooth margins to the lesion (arrows) and a large deep central "collar button" type ulceration (Letter A).

### Acknowledgment

This case is presented through the courtesy of Drs. Anthony Porcelli and Sheldon Alder, Good Samaritan Hospital, Suffern, New York.

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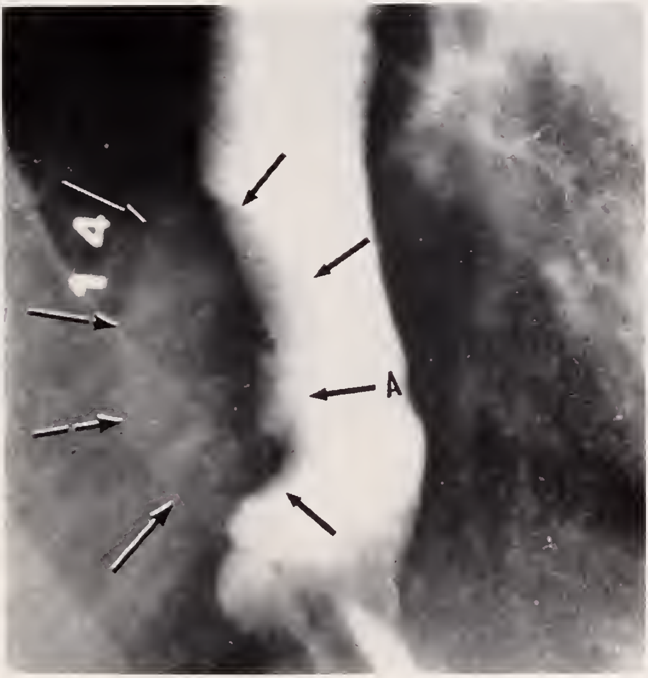
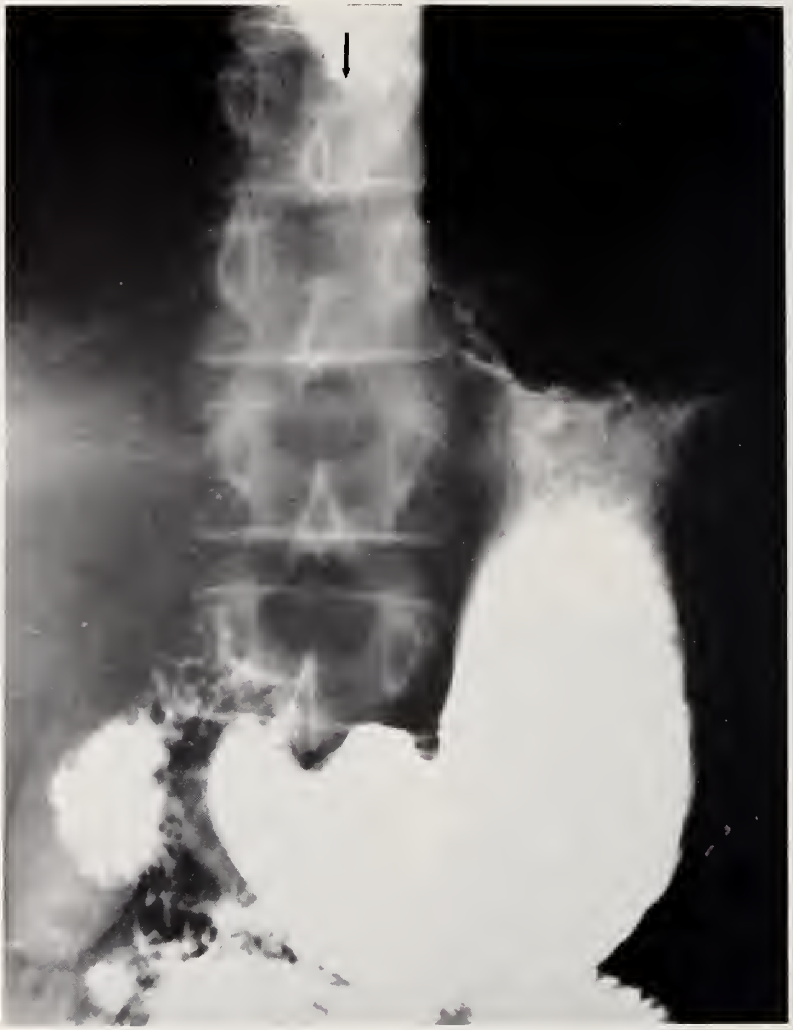
## CASE NO. 366

A 41-year-old man was admitted to the hospital because of epigastric pain, burning and eructations. There was no vomiting or bleeding. Complete blood count, routine laboratory examinations, vital signs, and physical examination were within normal limits except for epigastric tenderness. History included surgical repair of a hiatus hernia via transabdominal approach; previous radiographs and surgical details were not available.



Case 366, Fig. 1. Spot view of the lower esophagus and esophagogastric junction with the segment filled with barium shows a lesion of the right wall of the esophagus just above a small hiatus hernia. Both interior and exterior margins are delineated (arrows). A central depression with angular margins suggests ulceration (arrow A).



Case 366, Fig. 2. Antero-posterior erect view of the stomach shows a collection of barium held up in the lower thoracic esophagus (arrow). This barium outlines the superior margin of a submucosal mass in the lower esophagus. The esophagus is not dilated.

Gastrointestional series was performed. The lower end of the esphagus was abnormal. A smoothly outlined 4 cm lesion of the right aspect of the lower esophageal wall was present just above a small hiatus hernia (Fig. 1). There was an irregular depression in the surface of the lesion. In the erect position, a small amount of barium was trapped in the lower thoracic esophagus which outlined the superior surface of the mass (Fig. 2). A submucosal tumor such as a leiomyoma was diagnosed, with ulceration. At surgery, the mass was well circumscribed and noninfiltrating. The overlying mucosa was fixed to the mass and distorted but not ulcerated. The mass was "shelled out" without complication. The pathologist reported a benign leiomyoma.

### Discussion

As is well known, the right aspect of the esophageal wall is often delineated when a properly exposed film is obtained in a suitable subject. The esophagus is border-forming with the right lung throughout much of its thoracic course, being separated only by mediastinal and visceral pleurae and a variable amount of areolar tissue. Hence, a clue to the nature of pathology is available if one can determine the effect on the espohageal wall. In Figure 1, the entire soft tissue tumor, a leiomyoma of the esophageal wall, is precisely delineated; its interior margin bulges into the more dense barium column and its exterior margin is contrasted against the more lucent lung and possibly some medistinal areolar tissue (arrows). In addition to locating the lesion precisely, one can predict with certainty a well-circumscribed, noninfiltrating process.

The question of surface ulceration over such a lesion may be a difficult one to answer. Again in Figure 1, a central depression with angular margins suggested ulceration (arrow A). At surgery, however, the lesion was covered by intact mucosa, albeit fixed and distorted. Further, there had been no bleeding clinically, which commonly occurs when submucosal tumors ulcerate.

*Case Report:* LEIOMYOMA OF THE ESOPHAGUS

**Acknowledgment**

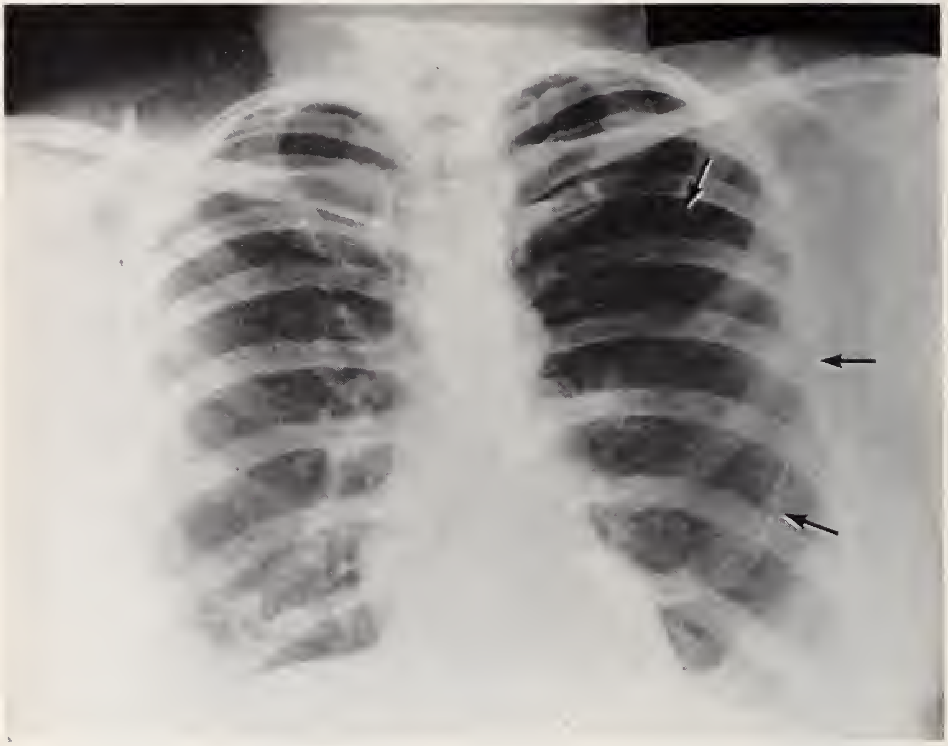
This case is presented through the courtesy of Drs. Allain Kaldany and Alfred S. Moscarella, Good Samaritan Hospital, Suffern, New York.

## CASE NO. 367

A 40-year-old woman was admitted to the hospital for gynecologic surgery, including dilatation and curettage, resection of urethral diverticulum, and vaginal repair. There were no significant complaints or findings other than those referrable to the pelvis. History was noncontributory; the patient was not certain about any provious chest x-ray examinations, and denied all symptomatology referable to the chest.

Routine physical and laboratory examinations were normal. Routine preoperative chest x-ray examination was performed, followed by multiple additional projections, expiration study, and tomography (Figs. 1–4).

In the frontal projection, there was a curvilinear density in the left hemithorax convex laterally running from apex to base which suggested the "lung line" of a partially collapsed lung with approximately 40 percent pneumothorax. Mediastinum and hemidiaphragm were in normal position with no evidence of abnormal intrathoracic tension (Fig. 1). Expiration study demonstrated mediastinal swing to the right and excellent symmetrical excursions of both leaves of the diaphragm (Fig. 2). Left lateral view demonstrated too many curvilinear lines to be explained solely on the basis of fissure and lung line (Fig. 3). Tomography demonstrated these lines to better advantage (Fig. 4). Fluoroscopy



Case 367, Fig. 1. PA chest (routine, inspiration) reveals a curvilinear density in the left hemithorax convex laterally which runs from apex to base (arrows). The appearance suggests the "lung-line" of a partially collapsed lung with approximately 40 percent pneumothorax. The mediastinal structures and the left hemidiaphragm are in normal position; hence there is no indication of a tension pneumothorax.

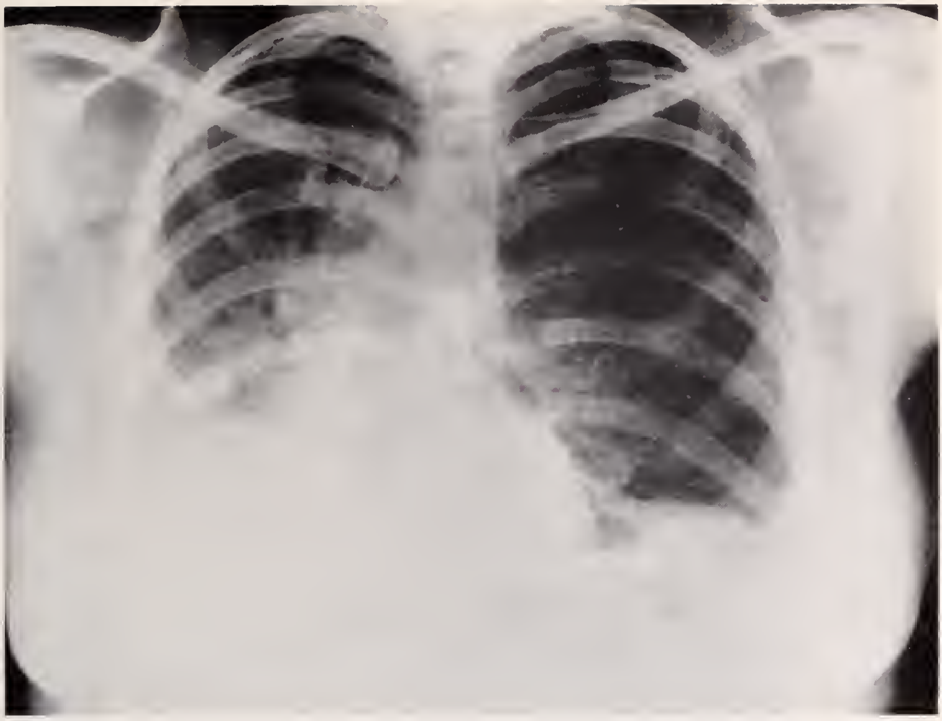
was not performed. It was concluded that there were multiple large bullae throughout the left upper lobe, although some additional free or loculated air in the pleural space could not be excluded entirely.

Patient was discharged after uneventful gynecologic surgery. She was readmitted six months later. Pulmonary angiography and surgery confirmed the presence of multiple bullae in the left upper lobe and lobectomy was performed.

## Discussion

Blebs and bullae are the common large air-containing cavities in the lung. By definition, a bleb is an air-containing structure located between leaves or layers of the visceral pleura, while a bulla lies within the lung parenchyma itself. Particularly when large, there are often no criteria for distinguishing point of origin or location; for simplicity, we will arbitrarily refer to the cavities as "bullae."

From the roentgenographic viewpoint, a bulla is recognized by its curvilinear



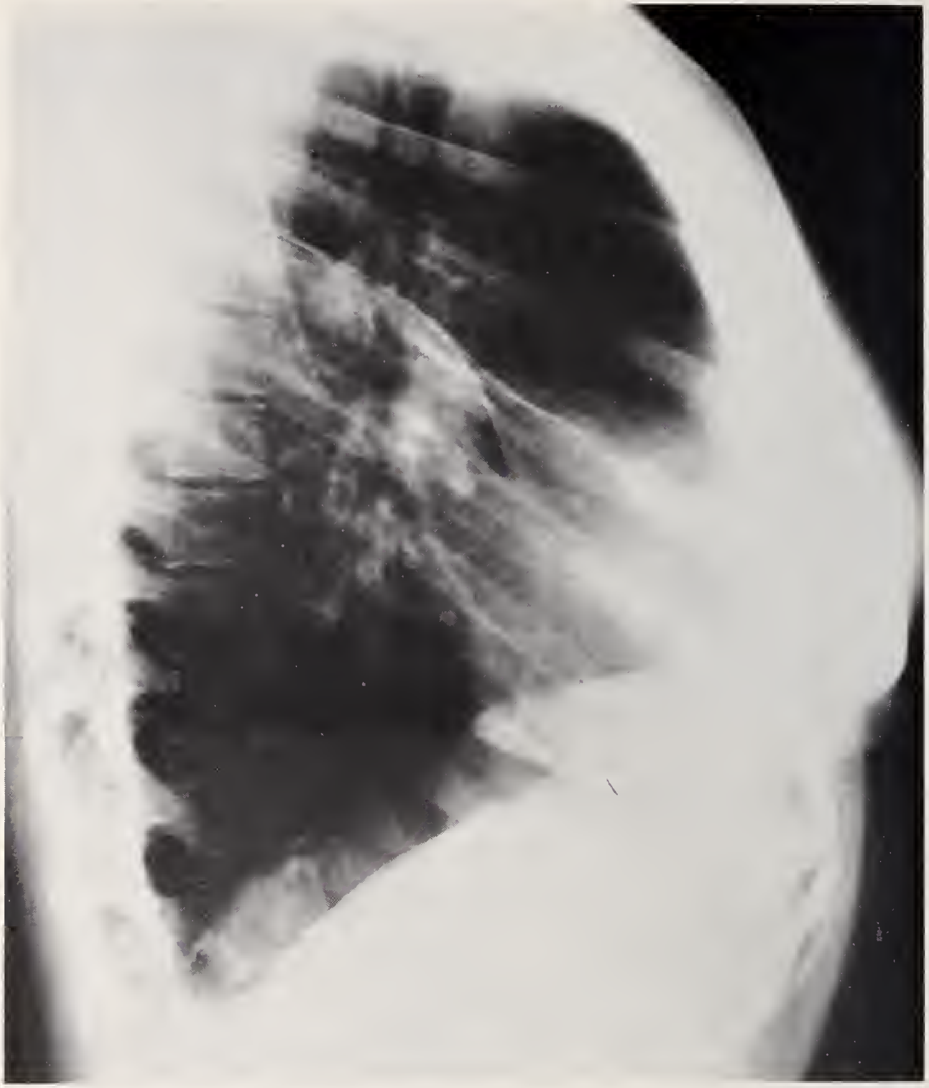
Case 367, Fig. 2. Expiration PA study shows mediastinal swing to the right along with excellent excursions of both leaves of the diaphragm. Some clouding of the left lower lobe is apparent as expected in expiration, but this is not as pronounced as on the normal right side.

margins along with the absence of normal bronchovascular markings and there is usually no problem in diagnosis. Occasionally, however, particularly when they are large or multiple, a problem in differentiating bullae from pneumothorax will arise, as in the case presented.

The easiest diagnostic aid used to clarify the presence either of air in the pleural space or a bulla is to expose a film in full expiration; the normal lung becomes more radiopaque relative to the radiolucent air and thus pneumothrax or bulla become more clearly defined (Fig. 2).

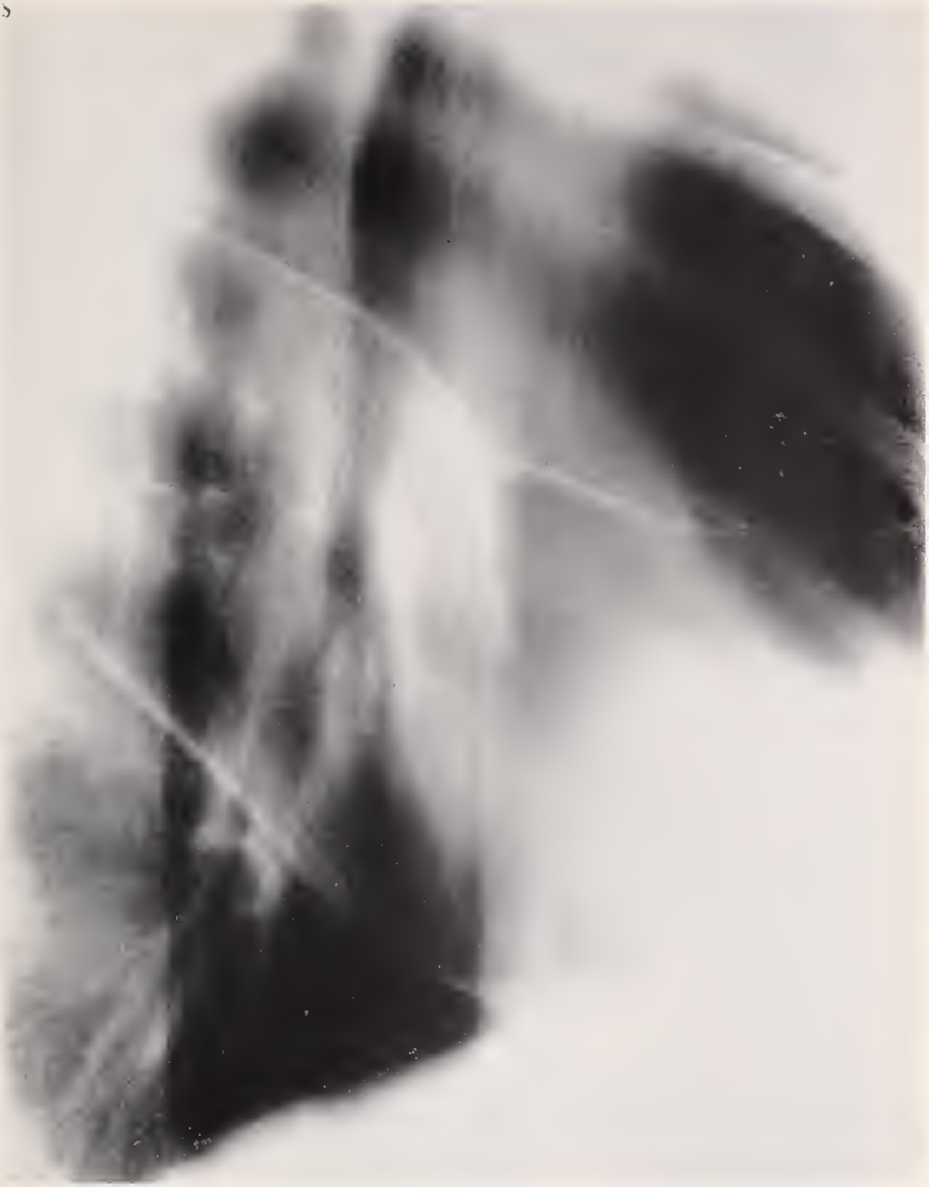
A second technique to establish the presence of air in the pleural space is to employ gravity with multiple decubitus projections and horizontal ray films, much the same in principle as one might do to establish the presence of small amounts of fluid in the pleural space. The technique was not employed in this case.

A third technique to establish the presence of bullae is to employ tomography (Fig. 4). Margins of the bullae which are virtually invisible on a conventional film may be easily and clearly defined on body-section radiography; further, the presence of additional unsuspected bullae may be discovered.



Case 367, Fig. 3. Left lateral view demonstrates a number of curvilinear densities whose number and location cannot be easily explained solely on the basis of fissure and lung line.

A note of caution in interpreting the significance of the contralateral mediastinal swing on the expiration film: this is not sufficient evidence to suggest the presence of a tension pneumothorax (Fig. 2). Any condition in which air is trapped and unable to be expired may result in abnormal dynamics in expiration; mediastinal swing or limited diaphragmatic excursion occur as the intrapleural pressure increases relative to the opposite normal side. The presence



Case 367, Fig. 4. Representative tomographic section through the left lung in lateral projection better demonstrates a number of curvilinear shadows.

of tension may be correctly inferred if mediastinal swing or diaphragmatic depression is noted on the inspiration film. These abnormal dynamics are often easily confirmed with fluoroscopy, not performed in this case. Finally, the combined presence of bullae plus pneumothorax, or the presence of loculated pneumothorax, may defy accurate roentgenographic diagnosis.

*Case Report:* PULMONARY BULLAE SIMULATING PNEUMOTHORAX.

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## CASE NO. 368

*Submitted by*

ROBERT KUHN, M.D.

A 41-year-old woman had diffuse swelling of the left ankle of many years' duration. Her earliest recollection of symptoms dated back approximately 25 years when she sustained several sprains of her left ankle within a period of several months. There were no significant symptoms until one year prior when she noted that her activities were becoming limited because of swelling and pain in the left ankle; symptoms were aggravated by all prolonged activities involving standing and walking.

Radiographic study of the ankle was performed in multiple projections (Fig. 1a–d). Numerous small, punched-out defects were noted in the tibia, talus, and inner aspect of the fibular malleolus. All defects were sharply circumscribed and exhibited thin surrounding margins of bone condensation. There was mild reactive sclerosis. The width of the joint cartilage was normal and mineralization was good. A diagnosis of pigmented villonodular synovitis was suggested and surgery was performed.

Arthrotomy of the ankle revealed masses of large, firm, discrete, irregular synovial tissue which were grasped and delivered from their beds in the anterior, medial, and lateral recesses of the joint. Projections of these masses had eroded through the articular cartilage and subchondral bone on all surfaces; punched-out defects were left upon removal of the synovial masses.

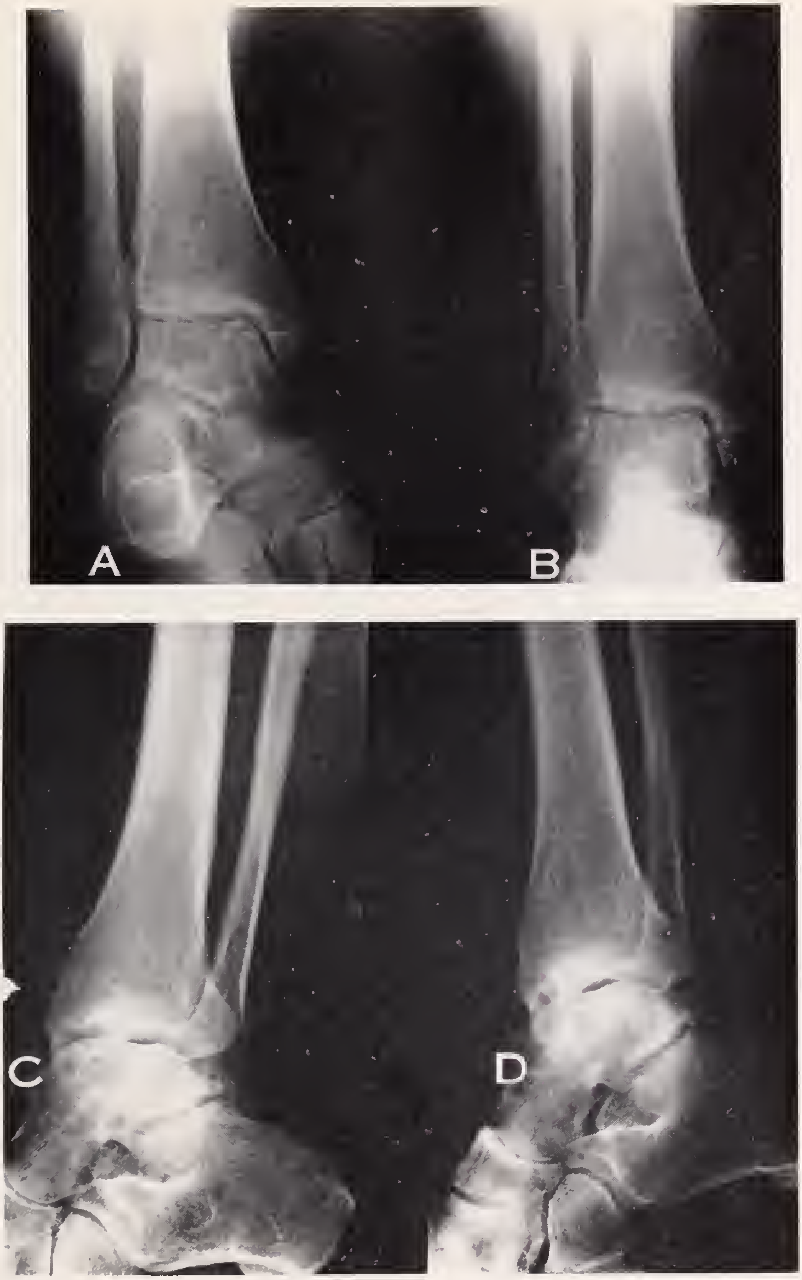
Three months after surgery the patients symptoms were about the same and the radiographic appearance was unchanged as well.

### Discussion

Pigmented villonodular synovitis (PVS) is a distinctive lesion most often occurring in the knee and more rarely in the hip, shoulder, and ankle; cases have also been reported in the small joints of hands and feet.

Pigmented villonodular synovitis usually develops in young adults but may go untreated until the 5th or 6th decade. It most commonly causes intermittent monarticular pain and swelling gradually increasing in severity with each recurrence. A history of trauma is usually obtained although a direct etiologic connecting is lacking. Experimentally produced pigmented villonodular synovitis in the dog has been produced by the repeated injection of autogenous blood into the knee. However, there is no greater incidence of pigmented villonodular

From the Department of Orthopedics, Good Samaritan Hospital, Suffern, New York.



Case 368, Figs. 1a–d. Multiple projections of the left ankle reveal numerous small lucent defects of varying size in the tibia, talus, and fibular malleolus. All defects are sharply circumscribed and exhibit a thin surrounding margin of bone condensation plus mild reactive sclerosis. Otherwise, the articulating surfaces are smooth, the joint cartilage width is normal, mineralization is good, and the ankle mortise is maintained.

synovitis in young athletes who have sustained repeated hemarthroses. It is likely that there is a predisposing factor present in certain individuals and that repeated trauma may be significant in producing the lesion.

Circumscribed and diffuse types of lesions have been described. In the circumscribed variety there are single or multiple sessile or tumor-like lesions as in the case described. The diffuse form involves the entire synovial lining with coarse villous and nodular outgrowths. The process may produce gradual erosions of articular cartilage starting at articular margins as a pannus-like growth. Tongue-like extensions may erode through articular cartilage and into the subchondral bone to form cyst-like cavities filled with the synovial growths. Extensions through the joint capsule may occur with destruction of the stabilizing joint ligaments. Bursae and tendon sheaths may also be involved.

Histologically, the synovium is composed of large polyhedral cells with the stroma containing fibrous tissue and hemosiderin pigment; varying amounts of hemosiderin determine to what degree the lesions will be pigmented. Multinucleated giant cells are frequent. This histologic picture was present in this case.

Treatment is by complete synovectomy, although recurrences are not uncommon. Diffuse lesions of tendon sheaths have been successfully treated by roentgen therapy.

*Case Report:* PIGMENTED VILLONODULAR SYNOVITIS OF THE ANKLE.

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ALEXANDER B. GUTMAN
1902–1973

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# In Memoriam

## ALEXANDER B. GUTMAN, M.D.
1902–1973

On Friday, May 4, 1973, Dr. Alexander Benjamin Gutman suddenly and without warning passed away. His family lost the dearest member, his friends their wisest counselor, his colleagues a standard bearer, his students a great teacher, the hospital a great leader, the school an energetic prime mover, and the world a distinguished scientist and extraordinary human being.

His career started with biochemical studies and continued with enzyme chemistry and its clinical applications and ended with studies of the molecular basis of gout. His intent was apparent at the outset when he took a Ph.D. degree in chemistry and experimental biology prior to his M.D. degree—and this a half a century ago. Each step of his career was characterized by an incredible determination, indefatigable industry, clarity of thought, analytic precision, and creative intelligence. Indeed, he displayed a rare combination of inductive and deductive reasoning. His productivity did not span a decade or two but stretched through five decades, during which time he ignored financial reward and the trappings of power. His directness of purpose and standards of excellence sometimes made him intolerant of others who shared neither these capacities nor goals. To some his reticence made him appear distant. He was not gregarious either socially or in his work. He worked alone or with selected and talented colleagues such as his long time coworker, Dr. Ts'ai-fan Yü.

Most men retire to less strenuous activity at an age when Dr. Gutman was still pursuing original investigation. Indeed, between 1970 and 1973, up until his death, he published, together with Dr. Yü, 22 papers concerned with the molecular basis of gout. Only his tragic and sudden demise put an end to this unbelievable effort.

It has been customary in evaluating Dr. Gutman's contributions to emphasize his role as an educator. In the age old struggle between the philosopher and the sophist, Dr. Gutman was in the camp of the former. He had a socratic disdain for material things and instant reward. In addition, consistent with his philosophic position was his continual search for truth and a desire to impart this to his students and colleagues. For this task he was armed formidably. His lectures were models of clarity and organization. His writing had a felicity of phrase and grace of style which were the envy of literary men, and his concern for the education of young people in medicine was never blunted by his own personal needs. While he trained many people to follow careers in research, his greatest interest and impact was in broader educational programs in the two medical schools with which he was primarily affiliated, and in the founding and development of the American Journal of Medicine ("Green Journal"). At the Columbia University College of Physicians and Surgeons he made his mark as a clinical investigator and as a teacher of fundamental pathophysiology. He was one of the pioneers who brought the laboratory to bedside teaching and patient care. Coming to Mount Sinai he accepted new challenges: to expand scientific effort in a hospital rich in clinical tradition and to plant the seed which ultimately flowered in the Mount Sinai School of Medicine. He functioned not only as Chief of Medicine of The Mount Sinai Hospital but also as the first Professor of Medicine of the new School. A full bibliography of Dr. Gutman's publications and a complete curriculum vitae may be found in the *American Journal of Medicine*, Volume 54, pages 693 to 696, 1973.

During all of this time he founded and nurtured single-handedly "The Green Journal" which became the leading journal of internal medicine in the world. He insisted on high literary and scientific standards. His innovations, such as symposia and seminars, have been widely imitated by other journals. Those of us who worked with him on the editorial board remember well his refusal to compromise the quality of the journal to appeal to a larger and less sophisticated audience. His interest also was manifest in his participation in the editing of and contributions to the Cecil-Loeb Textbook of Medicine.

What sort of a man was he? He was strong, physically and intellectually. He was the quintessence of the individualist of the 19th century. He abjured the petty bickerings of the groves of Academe. We live in an era which has seen the abandonment of a work ethic, the substitution of chicanery for honesty, of image-making for substance, of superficial understanding for profound insight, of consensus for individual decisions, of pragmatism for truth, of quick camaraderie for sustained friendship, of fractured language for elegant rhetoric. None of these substitutions was acceptable to Alexander Gutman. His modesty concealed a broad and far-ranging interest in areas other than medicine. His knowledge of art and literature was extensive. Since his college days, when he was a quarterback on the football team, he was an enthusiast for sport. He was an accomplished sailor and fisherman. In spectator sports, his tastes were catholic, but he preferred individualistic sports such as those of track and field. In his later years be became interested in horticulture and it was characteristic of him that he visited the home and gardens of Linnaeus in Sweden. His own garden reflected a labor of love, great effort and exquisite taste, and all this while producing great scientific work.

The gentle and witty side of Dr. Gutman was best appreciated by his close friends and family. He had a dry sense of humor and laughed loudest at jests made at his expense. He was gentlest of all to his lovely wife Daisy and his two grandchildren, Marco and Le-Al.

We have lost an original man, a scientist of the first rank, an educator par excellence, a human being with integrity second to none, a humanist, and a loving husband and grandfather.

Mortimer E. Bader, M.D.,
Richard A. Bader, M.D., and
Sherman Kupfer, M.D.
*for the*
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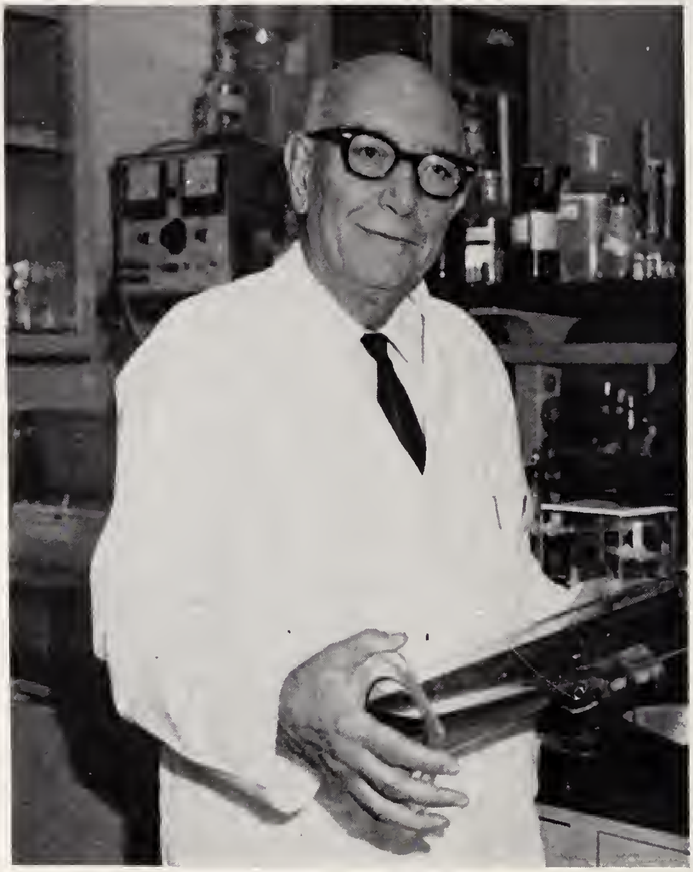

ISIDORE SNAPPER, M.D.
1889–1973

# In Memoriam

## ISIDORE SNAPPER, M.D.

## 1889–1973

The fabric of any institution is woven by the men who work there; thus, sometimes the imprint of the man upon the institution becomes indelible even if he had left many years ago and is no longer identified with it, and even if his basic philosophy runs contrary to that of the institution. A case in point is Dr. Isidore Snapper, who was Chief, Second Medical Service, and Director of Medical Education at The Mount Sinai Hospital from 1944 to 1952. These were the post-World War II years when crucial developments took place there.

Today, Snapper is a representative of a past era, a giant who was an admired example of that era. He reflected the excellence of the Old World, tempered by years in the Far East and in the United States. He combined better than anyone else supreme bedside management and clinical diagnosis with an inquisitive scientific mind. Helped by a memory of almost absolute recall, he developed both brilliant and challenging diagnoses and concepts. They could be confirmed only by detailed study and laboratory work that served as a stimulation for generations of physicians.

The universality of his approach contradicted modern specialization. To appreciate his unusual influence on a generation of physicians at Mount Sinai, his own development deserves to be sketched briefly.

Dr. Snapper was born and raised in Amsterdam, Holland, where, at age 22, he graduated with an M.D. degree and two years later obtained a Ph.D. in Groningen. There, working with two great men, the fledgling researcher put his lasting imprint on medicine. With Hamburger he clarified red cell permeability; and with van den Bergh he developed the bilirubin reaction which for generations remained the backbone of the differential diagnosis of jaundice. The young doctor had detected differences between the bilirubin crystallized from the serum of patients with hemolytic jaundice and that of patients with mechanical biliary obstruction.

At 30 years of age he became Professor of Medicine at the University of Amsterdam, something unheard of in Europe then and even now. While holding this prestigious position, he not only was a teacher much admired by his own faculty and students but also by physicians all over Europe whom he met while traveling extensively and whom he astonished by his fluency in many languages. During the Amsterdam years he was a prolific contributor to journals covering many different fields, and almost every volume of the prestigious *Biochemische Zeitschrift* had one of his articles.

Well trained as he was in physiological chemistry, he studied the metabolic function of the kidney and laid the foundation for the modern concept of conjugation, particularly with glucuronic acid. It is one of those paradoxes of medical progress that even the intellectual master, Snapper, who, with van den

Bergh, was responsible for the separation of direct from indirect bilirubin and who clarified glucuronic acid conjugation, would have to leave it to others to connect these two phenomena almost thirty years later.

In addition to his biochemical activities, Dr. Snapper is credited with many other original observations and identifications of disease entities, such as the description of Boeck's sarcoid, which he called "pseudotuberculosis," and that of eosinophilic granuloma. Bone diseases became his particular "hobby," and he contributed importantly to their metabolic aspects. Eventually he became concerned both with the laboratory and clinical aspects of all facets of internal medicine. There are few men who have mastered many fields simultaneously and also contributed original work in all of them. He was a forerunner of the present clinical scientist, the internist known for his laboratory achievements.

Honors came from all sides as he traveled widely, and when this writer first met him in the middle 1930s, his mastery of metabolic clinical knowledge was virtually unequaled in Europe. His travels were not only occasioned by the invitation of the medical fraternities in the various countries who desired to listen to his exciting presentations. Rather, he journeyed more often as the most famous soccer referee in Europe who combined the opportunity to referee an exciting international game on Sunday afternoon with lectures on the preceding Friday evening and ward rounds on Saturday. Dr. Snapper told the story that the insults which the excited crowd hurled at the referee counterbalanced the arrogance which a young Professor acquired in the hierarchical environment of European medicine.

He decided to move from the then safe-appearing Holland to this country in 1938, at a time when no one expected that Hitler would overrun the Netherlands. Indeed, this move was at that time considered the peculiar behavior of an adventurous Professor. After settling his children here, he went to Peiping Medical College in China for three productive years where, apparently, his main interest shifted from the laboratory to the bedside. He was captured by the Japanese, exchanged for a Japanese General held by the Dutch, and came to this country where he served as consultant to the Office of the Surgeon General of the Army before coming to Mount Sinai in 1944. The eventful eight years in our institution, while he was also Clinical Professor of Medicine at Columbia, will be better remembered by most readers than by this writer who had the good fortune to be together with him subsequently at Cook County Hospital in Chicago. There, as Director of Medical Education, he fascinated the well-known institution by his presentations. He became interested in pathological anatomy, and his performances at the daily pathologic and weekly clinical pathologic conferences became legendary all over the Middle West because of his charisma and unbelievable reservoir of knowledge.

In 1953 he returned to New York where, for 12 years, he was at the Beth-El Hospital, subsequently called Brookdale Hospital, and for the last eight years was Consultant-in-Residence at the Brooklyn Veterans Administration Hospital. He was a leader and innovator in both institutions. Up to his death he remained a prolific writer of articles and books. The last two on "Myeloma" and "Bedside Medicine" appeared in several editions as standard works—gems to read.

As he grew older he added a historical outlook to his writing concepts, but he remained a brilliant diagnostician, the sought-after consultant with a font of information, and sustained an almost compulsive curiosity for all aspects of clinical medicine. Virtually up to his death a phone call from him with a pointed question remained a stimulation.

Together with his many students all over the world, with his wife, children, and grandchildren, the Mount Sinai community mourns his demise. We have lost one of our giants whose eight short years at this institution left such impact. His colleagues and students will speak of him with awe and respect for years to come as a man for whom the word "charisma" might have been invented.

HANS POPPER, M.D.
*for the*
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ALFRED E. FISCHER, M.D.
1900–1973

# In Memoriam

## ALFRED E. FISCHER, M.D.

1900–1973

Alfred E. Fischer, Consultant Pediatrician at The Mount Sinai Hospital, died at his home after a long illness on April 7, 1973 at the age of 73.

Alfred Fischer was born in New York. His father, Dr. Louis Fischer, was one of the pioneer pediatricians in this city and was the author of the popular book, *The Health Care of the Baby*. Alfred received his A.B. degree in 1919 and his M.D. degree in 1923 from Cornell University. He served as intern and house officer at Mount Sinai from 1923 to 1925 and thereafter as resident physician at the Willard Parker Hospital. Following postgraduate studies in Berlin and Vienna, he returned to Mount Sinai as resident pediatrician.

In that era at Mount Sinai, the tradition included an active participation in research as well as clinical service. Throughout his career, Alfred participated in investigative studies while maintaining a busy private practice. He was advanced from Adjunct Attending at Mount Sinai to Associate Attending Pediatrician and eventually to Consultant Pediatrician. He also served as Visiting Physician at the Willard Parker Hospital and was Consultant Pediatrician to the Monmouth Memorial and the Fitkin Memorial Hospitals in New Jersey.

Dr. Fischer organized the first clinic at Mount Sinai for the treatment of diabetic children. He was a former president of the New York Diabetes Association and helped to establish Camp Nyda, the first summer camp for diabetic children in this area. He published many papers related to the genetics and the management of diabetes in children including *The Handbook for Diabetic Children*. With the establishment of an obstetrics service at Mount Sinai, his most recent contributions were concerned with the clinical features and the management of the infants born to diabetic mothers.

During his association at the Willard Parker Hospital, which was the principal contagious disease center in New York, he was actively engaged in research studies as well as with the teaching of students from several of the medical schools. He held an appointment as Assistant Clinical Professor in Pediatrics at the New York University Medical School. Investigative studies in poliomyelitis were the subject of many of his publications and he was among the first to note the relationship between tonsillectomy and bulbar poliomyelitis. These studies led to the ban on the performance of tonsillectomies during the summer season. He authored many other publications related to the treatment of pertussis and measles.

Dr. Fischer participated actively in many professional organizations. He was a Fellow of the New York Academy of Medicine which he served as chairman of the pediatric section and the admissions committee, and as a member of the executive committee on public health relations. He was a Fellow of the American Public Health Association and a member of the American Diabetes Association.

He was a Fellow of the American Academy of Pediatrics and served as a district representative to the national executive committee. He was appointed to the pediatric advisory committee of the New York Health Department and had been chairman of the committee on child welfare of the New York County Medical Society.

With all of his professional commitments, Alfred somehow found the time to provide his services to community projects. He was a member of the medical board of the New York Guild for the Blind and he served as a trustee and pediatric advisor to the Jewish Child Care Association.

I was privileged to have been a close friend and associate in the practice of pediatrics for over twenty-five years. He possessed the rare gift of the outstanding clinician of being able to intuitively sense the nature of a child's illness. He was dedicated to his patients and was rewarded by their love and gratitude. He was devoted to his wife, Selma, to his daughters, Ellen and Susan, and to his three grandchildren. He was a warm and generous man. We shall all miss him.

RALPH E. MOLOSHOK, M.D.
*for the*
EDITORIAL BOARD

# The Implications of Hyperammonemia in Rare and Common Disorders, including Migraine

ALEXANDER RUSSELL, M.D.

*VII.* Before embarking upon its implications in so common a disorder as migraine, I would like to touch upon three less common situations of no little importance.

### *A. Reye's Syndrome*

A clinico-biochemical reappraisal of the peculiarities of CNS disturbance in the syndrome described in 1963 by Reye and coworkers as an encephalopathy with fatty infiltration of liver and kidney has led Huttenlocher to suggest an underlying hepatic or rather hyperammonemic encephalopathy. They were able to vindicate this view in each of three consecutive cases by revealing intense hyperammonemia during the acute phase of the illness.

Typically, the child appears at the outset to be recovering from a mild febrile illness or prodrome, presumably of viral etiology, expressed in little more than cough, rhinorrhea, or mild gastrointestinal disturbance. Repeated vomiting then supervenes, cerebral dysfunction manifesting itself within a further 24 to 48 hours. A brief phase of delirium may transiently precede lethargy and coma in older children. Convulsions may then appear, and in comatose patients, bilaterally symmetrical rigidity or spasticity is characteristic, alongside hyperactivity of tendon reflexes, sustained ankle clonus and extensor plantar responses —in other words, and in this context, typical clinical parameters of hyperammonemia.

Associated disturbances in respiration are a special characteristic and are usually linked to respiratory alkalosis. Other evidence of brain stem dysfunction may appear, including ocular palsies and loss of pupillary responses. The syndrome may advance rapidly in the most serious cases, with a fatal outcome likely. In survivors, however, stupor and coma may have lasted up to 48 hours. Although the CSF is normal, the EEG is usually grossly abnormal, with high voltage delta activity. Liver dysfunction is indicated by exceptionally high SGOT levels, raised cephalin flocculation, but lowered prothrombin time. Gastrointestinal bleeding has occasionally ensued. But the most striking feature is the intensity of the hyperammonemia from the very outset. In one child, 542 $\mu$g/100 ml was the level, in the other 762 $\mu$g/100 ml, and in another reaching 1,027 $\mu$g/100 ml.

In the third case, a three-and-a-half-month-old male who succumbed, astrocyte nuclei in the cerebral cortex were swollen, especially in the areas of neu-

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Professor and Head, Department of Pediatrics and Child Care, Hadassah University Hospital and Medical School, Hebrew University of Jerusalem.

This paper was presented as the Bela Schick Memorial Lecture of The Mount Sinai Hospital on October 17, 1972. The first part of this paper was published in the previous issue, September–October 1973.

ronal necrosis. Early stages of Alzheimer Type II changes may have already been represented.

There is little doubt now, therefore, but that we are dealing with a hepatic encephalopathy, with an acute ammonia intoxication stemming from fulminant liver pathology and evoking a predominantly hyperammonemic symptom complex marked by stupor to coma, linked to typical neuropathic features. Alertness to this clinical component may well be life-saving since an opportunity then arises for rational management in the hope of tiding the patient over his acute crisis. Cleansing and neomycin enemas, intravenous hydrocortisone, intragastric neomycin, etc., should be provided. Whether therapeutic intervention with aspartic acid along the lines we later propose would be more effective remains for clinical trial.

With over a hundred recorded cases having accumulated since the original report of 21 cases from Australia, we can include this distinctive entity among relatively common postviral disorders.

### *B. Hyperammonemic Mental Retardation and Cerebral Atrophy of Rett*

Rett (1966) and Stockl (1968) claim to have unearthed varying degrees of unexplained hyperammonemia in 39 girls during a survey of 6000 retarded children of both sexes. Fifteen of these also suffered convulsions, and five died. In a personal communication we learned something of the findings in the brains examined by Seitelberger and his colleagues. A tendency toward cerebral atrophy with mild neuronal loss, a "spongy" cortex, and myelin breakdown were noted. Although there was astrocytic proliferation, no reference was made to specific Alzheimer glia. Indeed, their absence would not be surprising since no blood ammonia estimation ever exceeded 165 $\mu$g/100 ml in which the normal mean level is given as 86 $\mu$g/100 ml. Whenever such cells have been identified, the corresponding blood ammonia levels prevailing at the time have ranged from 350 to 1000 $\mu$g/100 ml. No enzyme studies were undertaken; the pathologic significance of some ammonia results remain in doubt, and the clinical character of the cases is too heterogeneous to leave a convincing impression of any valid entity within this group.

### *C. Iatrogenic Hyperammonemia in the Newborn: Intravenous Amino Acid Alimentation*

We can turn at this point to some of the commoner situations in which it has thus become possible to at once identify the crucial concealed element of hyperammonemia upon which hinges survival of the patient. For instance, premature and full-term infants undergoing intravenous alimentation with casein or fibrin hydrolysates were sometimes found to manifest undue drowsiness even to the extent of stupor and coma. Neuropathic features might appear, including muscle twitching or even convulsions, which could easily be ascribed to the preexisting condition for which they were being treated. We must appreciate, however, that the dominant lethargy, hyperreflexia, etc., might well be indicative of underlying ammonia intoxication stemming from inability of the immature liver to adequately dispose of the ammonia derived from these concentrated amino acid preparations. Johnson et al (1972) have also recently confirmed this suspicion by finding pathologic levels of blood ammonia ranging from 86 to 304 $\mu$g/100 ml in many infants being treated in this way. There is also the interesting finding of Stegink and Baker (1971) of low plasma arginine values in infants receiving casein hydrolysates which may in part reflect incompetence or failure of the Krebs urea cycle. This observation recalls the intensification of symptomatology, even to the extent of crisis, when the child suffering from inborn urea cycle defect is given similar casein hydrolysates such as Nutramigen.® Liver dysfunction was identifiable in four of those seven infants, at least in terms of high SGOT levels (115–306 units), in three of which they were restored to normal after withdrawal of the hydrolysates. This constitutes a valuable and timely warning to carefully avoid iatrogenically induced coma in the newborn. Meanwhile, we recommend that physicians initiate such therapy with a low concentration of protein hydrolysate (1.0–1.5 mg/Kg per day), progressing to higher concentrations only when the blood ammonia concentration and other liver function tests are normal. It would be reasonable, furthermore, to request an amino acid composition more closely approximating that of newborn plasma and from which the ammonia has been removed. Apart from compound amino acid preparations that can be manufactured and stored under strict control in one's own laboratories, FreAmine is commercially available and contains only 600–700 $\mu$g ammonia nitrogen per 100 ml. Cutter Aminoacid Solution,® which is still available only for investigational use, contains 300–400 $\mu$g ammonia nitrogen per 100 ml. Such concentrations of $NH_3$ represent one- to two-hundredths of those found in protein hydrolysates.

## *VIII. A CLINICAL EXPRESSION OF A HETEROZYGOUS STATE, AND ITS IMPLICATIONS IN THE MIGRAINE AND CYCLICAL VOMITING OF CHILDHOOD*

Finally, we humbly approach the implications of hyperammonemia in the commonest of the disorders to confront us today: migraine, along with its childhood equivalent, cyclical vomiting.

Certainly, migraine is one of the commonest disabilities of man, so common that you may be scornful of it—unless you yourself suffer its recurrent anguish. A distinguished British neurologist and friend, Henry Miller, places its incidence as high as one in ten, so that in the United States alone, there must be over ten million adult victims. The figures in childhood have been lower, fluctuating between 2.0 and 4.6 percent, which may be nearer the valid adult incidence. *A.* We have already recognized recurring migrainous components in the homozygotes of the inborn urea cycle defects described. And, as I have already mentioned, my original Case 2, homozygous for OTC defect, had indeed been authoritatively diagnosed as ophthalmoplegic migraine. The simple, heartfelt comment by her maternal aunt, mother of Case 1, when the latter infant was 20 months old, had provided my first clue to a possibly comparable, if not identical, clinical expression of the heterozygous state when it is subjected to appropriately specific stress: "I have been ill," she said, "ever since the conception of my child. . . ." She then related the continuous thread of migrainous headache and vomiting from almost the outset of pregnancy, although with apparently cyclical intensifications, and with frequent incidents of vertigo and also true fainting, as an especially disabling accompaniment.

It was, therefore, the appreciation of continuously severe upset throughout the mother's pregnancy with the homozygous first OTC case that initially alerted me to the possibility of a distinct clinical expression of this heterozygote state as migraine. Indeed, the mothers of both children experienced a similar pattern of overwhelming prenatal illnes, but only during their pregnancies with the affected children, and also in each, during that pregnancy which had ended in neonatal death. Moreover, both mothers had themselves been subjects of severe migraine since childhood, as had their own mothers and maternal grandmothers before them. Subsequent proof of underlying hyperammonemia in the progeny surviving these stormy pregnancies reinforced the suspicion that at least the maternal migraine during pregnancy could be a clinical expression of their prenatal intolerance to "fetally derived" ammonia excess. If one presumes that both mothers are truly heterozygous for this trait and are, therefore, enzymatically inadequate in the handling of ammonia, those high concentrations reaching them from the affected progeny would suffice to induce hyperammonemic symptomatology compatible with severe migraine.

### *B. Ammonium Tolerance and Enzymologic Screening of the Heterozygote Trait*

It was a logical step, therefore, to next subject both mothers to the ammonium tolerance test we were evolving (Fig. 16). After several placebo "loads" had produced negative results, an oral dose of ammonium chloride was given. Within the hour in one mother it produced the worst attack of migraine she had ever suffered, while in the other mother the reaction was deferred for 4 to 5 hours, whereupon she awoke from an attempt to sleep off her oncoming headache with nausea and vomiting leading to fainting (precisely as it had done repeatedly during her last pregnancy). Furthermore, vision was totally lost for several hours, happily to return, quadrant by quadrant, simulating migraine in reverse. When related to parallel changes in blood ammonia we can see how seriously toxic elevations of blood ammonia had followed the small loading doses of ammonium chloride—the peak thereof coinciding with the onset of headache in the first mother within the hour; the reaction being delayed for several hours, albeit severe enough, in the second mother.

Incidentally, the identical twin of Case 2, who was not to manifest migraine for a further two years, although she was later to be accepted on enzymatic grounds as a heterozygote, already presented a clearly abnormal "premonitory" curve at this point.

Subsequently, even when relatively small protein loads were administered instead, for example, as little as one egg in this test, a comparably abnormal curve of blood ammonia could be demonstrated, not only both in heterozygotes and

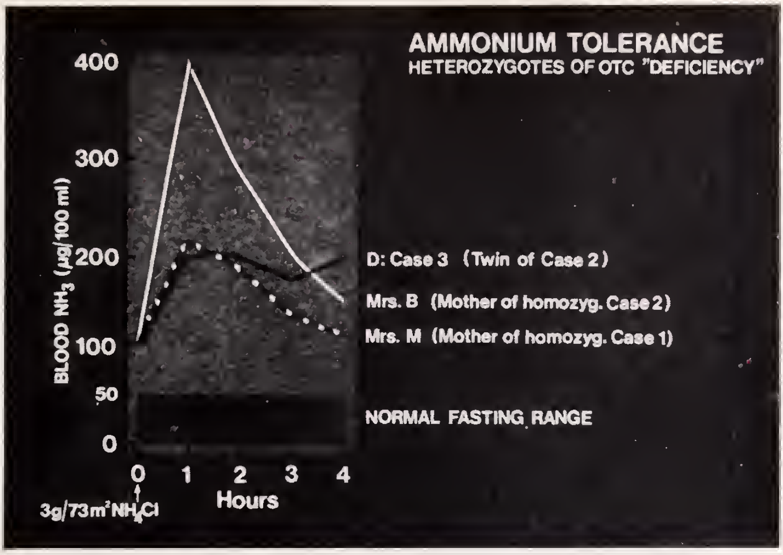


FIG. 16. OTC-ase defect: Ammonia tolerance testing of both mothers (of homozygous Cases 1 and 2) and Case 3—all presumptively heterozygous. In mother of Case 2 (Mrs. B.), an extreme blood ammonia peak of 400 µg/100 ml coincided with perhaps her most intense attack of migraine. In Mrs. M., although the onset of migraine followed several hours after load, it was equally devastating, even culminating in transient blindness.

homozygotes of OTC deficiency, but also in the heterozygotes of ASA whom we studied (Fig. 17).

The next step involved attempts at enzymatic assay in these presumptive heterozygotes. Liver biopsy is not exactly a welcome procedure in heterozygote study, so we first turned to jejunal biopsy at my request, and later even to skin scrapings from the heel. Jejunal material had already proved to be a suitable source of urea cycle enzyme activity. The jejunal mucosa of the mother of OTC Case 2, and skin material both from the mother of Case 1 and from the second twin then revealed their heterozygous status for this trait, with approximately 50 percent defect of activity of OTC in each.

### *C. Study of Migraine and of Cyclical Vomiting as a Migraine Equivalent in Childhood*

Thereafter, my research fellows, Margaret Davidson, Myra Meshulam, and I pursued ammonia and protein tolerance testing beyond this pedigree, and in a series of relatively well-defined examples of juvenile migraine, of both severe and mild degree. Classically, migraine embodies a basic triad (Fig. 18): recurrent paroxysms of headache, most often unilateral and frontal, linked to premonitory and/or concomitant ocular aberrations, together with nausea, often culminating in vomiting.

Such a migrainous basis is infrequently appreciated in so-called cyclical vomiting, a well-worn entity of pediatrics often fondly relegated to the nebulous realm of the "periodic syndrome." The intensity and intractability of the vomiting dominates the clinical picture to such an extent that the headache as well as the lethargy, even when it may actually amount to stupor, usually comes to be interpreted as merely incidental to alleged ketosis and dehydration. The opportunity does not often come the way of the pediatrician to trace the whole

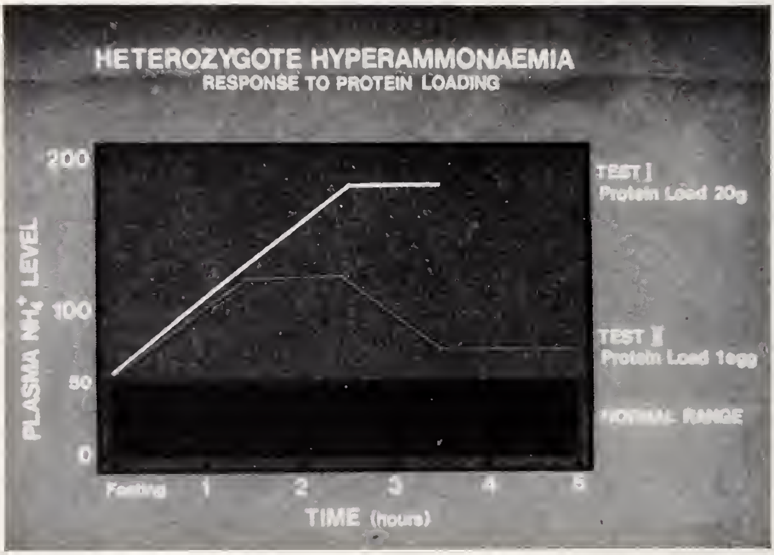
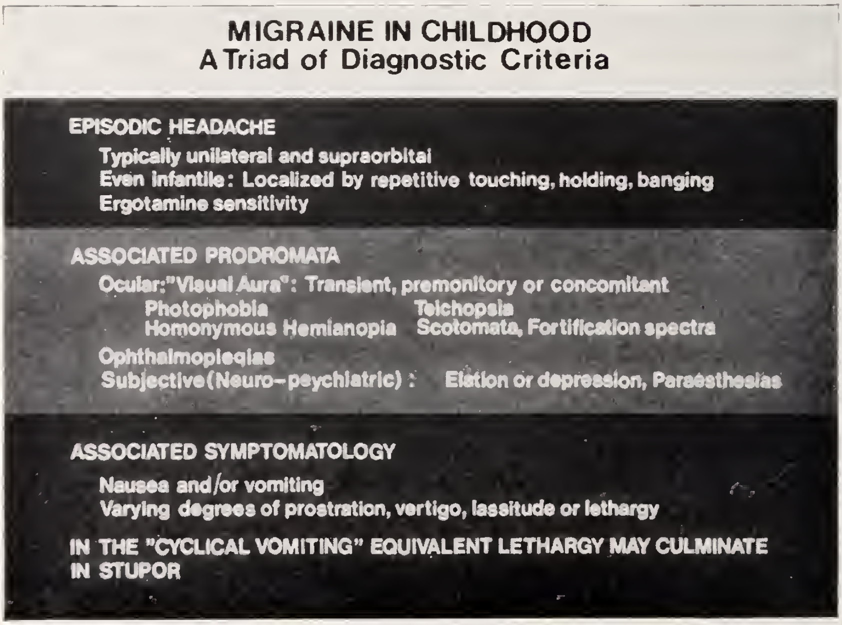


Fig. 17. Protein tolerance tests on Case 3, regarded as heterozygous, belatedly manifesting first episode of migrainous "cyclical vomiting" at age of nine years. Abnormally high peaks of blood ammonia after relatively small protein loads.



MIGRAINE IN CHILDHOOD
A Triad of Diagnostic Criteria

EPISODIC HEADACHE
Typically unilateral and supraorbital
Even infantile: Localized by repetitive touching, holding, banging
Ergotamine sensitivity

ASSOCIATED PRODROMATA
Ocular: "Visual Aura": Transient, premonitory or concomitant
Photophobia — Teichopsia
Homonymous Hemianopia — Scotomata, Fortification spectra
Ophthalmoplegias
Subjective (Neuro-psychiatric): Elation or depression, Paraesthesias

ASSOCIATED SYMPTOMATOLOGY
Nausea and/or vomiting
Varying degrees of prostration, vertigo, lassitude or lethargy

IN THE "CYCLICAL VOMITING" EQUIVALENT LETHARGY MAY CULMINATE IN STUPOR

Fig. 18. Migraine: An outline of diagnostic criteria.

natural history of cyclical vomiting, beginning with its gradual and typically postpuberal evolution into the more classical configuration of migraine. The strong family history of migraine so often elicited should, in any case, more promptly identify many of the preliminary episodes of cyclical vomiting for the juvenile equivalents of migraine that, more often than not, they are.

On these grounds and on the premise that cyclical vomiting (the clinical sequence of which also includes headache, malaise, anorexia, nausea, vomiting, and lassitude to lethargy) and its periodicity (which could imply a gradual and recurring buildup in protein intake and thus ammonia intoxication) might well be related to a similar biochemical disturbance. We have especially focused attention upon cases of this type. In other words, we have in the main concentrated upon severe examples of childhood migraine, especially where these are also characterized by sufficiently intense vomiting and lethargy to justify description as cyclical vomiting.

To-date, fifteen such well-defined cases have been subjected to ammonia and protein loading. Nine have shown abnormally high basal (or fasting) blood ammonia levels in the range of 70 to 120 $\mu$g/100 ml. (Normal range for our microscale modification of Fenton's method being 10 to 45 $\mu$g/100 ml.) The ammonia and protein loading tests have, moreover, been positive in 8, with peak blood concentrations in the abnormal range of 130–180 $\mu$g/100 ml which have been directly correlatable in 6 with migrainous symptomatology. The maximal blood ammonia levels, and the symptom sequence, have also been correlatable with characteristic EEG modifications in at least 7 cases. Slow waves of the theta band (4–5 cps) were outstanding, and paroxysmal bursts of slow theta waves accentuated. A significant proportion of less intense and so-called "mild" cases of juvenile migraine have also displayed *basal* blood ammonia levels just above the upper limit of the normal range, but still with abnormally high peaks after loading, ranging between 80 and 115 $\mu$g/100 ml.

Of 7 cyclical vomiters subjected to enzyme assay, 3 have proven to be heterozygotes for the OTC defect. Of special interest, in addition, is a recent case where an approximately 50% deficiency of CP synthetase has been defined in liver biopsy material. The implications of this latter finding are important in so far as heterozygotes for defects of any of the other 3 urea cycle enzyme steps might equally well prove to have clinical expression as migraine.

Evidence for the genetic transmission of migraine, and even of cyclical vomiting, has long been impressive. Indeed, repeated acknowledgment of migraine as a strongly heredo-familial disorder can be dated back as much as 1900 years to Aretaeus who documented the first classical description of paroxysmal "hemicrania" and vomiting. His account even spoke of "much torpor"—the marked lethargy which is such a frequent characteristic of severe migraine and especially of the cyclical vomiting, but has been so often forgotten since. A more recent item in support of the genetic basis of migraine was the study of fifty of its child victims by Krupps and Friedman in 1959: 84 percent of the parents suffered intense recurrent headache which was classic migraine in 70 percent. Ample evidence elsewhere has supported this theme, but what precisely was the inborn biochemical trait being transmitted remained unknown.

### *D. Therapeutic Approaches*

Obviously, in hyperammonemic cases thus elucidated, a rational approach to the study of specific treatment, or preferably of prophylaxis, becomes feasible. Our explorations have focused upon various methods of reducing protein intake on the one hand, or augmenting ammonium disposal on the other. In the more severely affected homozygotes, lowering of the daily protein intake below 1.0 gm/Kg/day suffices to swiftly depress the toxic levels of blood and CSF ammonia to levels nearing normal, if only at the price of a subminimal protein intake below the level essential for normal health and growth. Alternatively, efforts to augment ammonium disposal have been concentrated upon the tricarboxylic acid cycle and its subsidiary $\alpha$-ketoglutarate-glutamic acid-glutamine system, a crucial mechanism for ammonia binding. Bessman has postulated depletion, primarily of $\alpha$-ketoglutarate, secondarily of the whole Krebs tricarboxylic acid cycle, as a basis for the neuropathic toxicity of ammonia through interference with aerobic glycolysis and cerebral oxygen consumption as the main elements.

Although as yet unproven, this hypothesis was in part subjected to therapeutic test in our next approach. In turn, supplements were tried of two substrates of the tricarboxylic acid cycle, initially citrate and then aspartate, the goal being an enrichment of one or the other cycle substrates and thence reinforcement of $\alpha$-ketoglutarate and a possible induction of its increased conversion into glutamine. Indeed, citric acid administration undoubtedly "lowered the blood ammonia" significantly in the heterozygotes as well as in both OTC homozygotes. In one example of cyclical vomiting given an ammonium chloride load which produced a rise of blood ammonia (Fig. 19), a simultaneous single dose of citric acid reduced this rise appreciably, and without any increase of urinary ammonia excretion. The citric acid effect, however, neither seemed consistent enough, nor sustained for long enough, after the initial promising therapeutic responses. Nonetheless, a prophylactic trial has been encouraging in two severe cases of classic cyclical vomiting, where convincing clinical control with significant reduction of the frequency and intensity of attacks, has followed the long-term use of 2 gm/day of citric acid. A subsequent case of greater clinical severity showed a more dramatic effect with administration of aspartate (Fig. 20). The base-line of a protein load of 25 gm produced an increase of blood ammonia to over 115 $\mu$g/100 ml. After 5.0 gm of citric acid there was limited curtailment of the abnormal rise. When, instead, an aspartate supplement was given simultaneously with the protein load, the abnormal rise in blood ammonia was actually averted, even when the protein load was doubled.

Obviously, where biochemical analysis of a migrainous subject, which we now regard as his right, has revealed other specific heterozygous or even homozygous defects, long-term management is to be modified accordingly. In the fa-

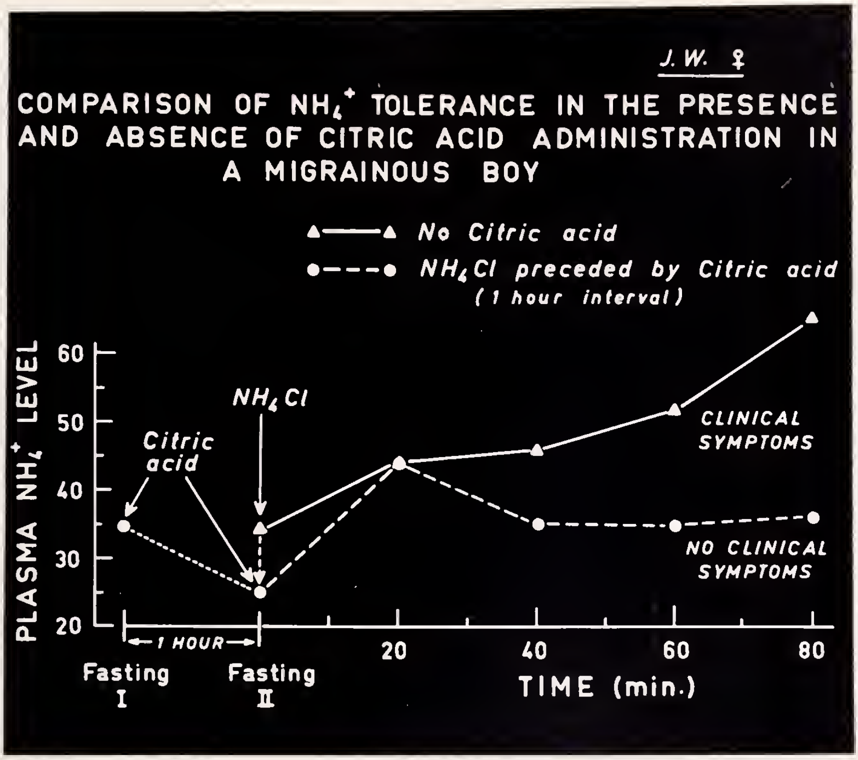


FIG. 19. Trial of citric acid in therapeutics of hyperammonemic migraine.

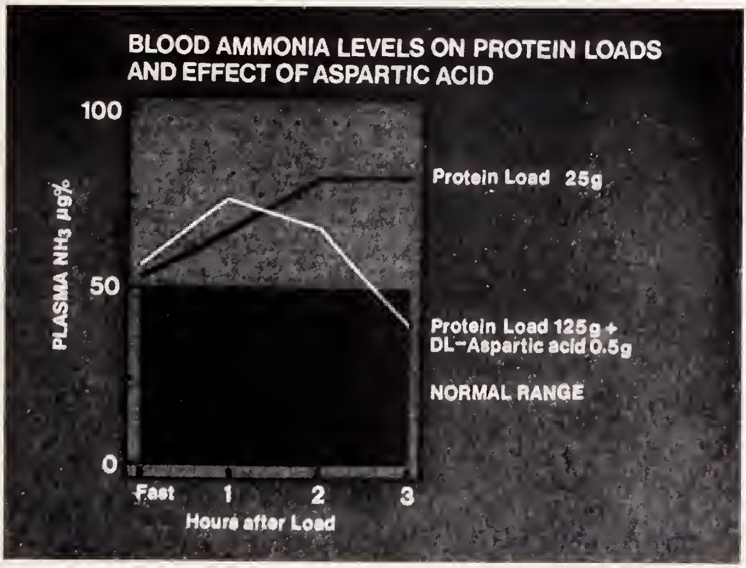


FIG. 20. Aspartic acid in hyperammonemic migraine: Lowering of blood ammonia elevation after protein loading when simultaneous aspartic acid supplementation introduced.

milial protein intolerant, for example, daily maintenance on an arginine or ornithine supplement would be necessary to circumvent the defect and avoid its clinical expression with headache, vomiting, and so forth.

All these findings are certainly of great interest in demonstrating at least one possible biochemical basis for some classic examples of migraine or of cyclical vomiting in childhood. As we have said, it has long been known, perhaps for 1900 years, that there is a strong hereditary basis for migraine. But what underlies the inherited trait is not known. In some examples of migraine and cyclical vomiting, therefore, the inborn element is an underlying heterozygote defect: deficiency of OTC, or of CP synthetase, if not of other enzymes of the Krebs-Henseleit urea cycle, or hypothetically of any other inborn defect leading to functional disturbance of the Krebs urea cycle. For instance, in familial protein intolerance, perhaps even consistently in cystinuric homozygotes, depletion of ornithine and arginine via the extrahepatic defect will interrupt its overall function. The trigger factor would appear to be a single or cumulative protein stress.

### *E. Conclusion*

We talked first of the dread cerebral and other implications of the constantly intense ammonemia in the homozygotes of these rare inborn urea cycle enzyme and related defects, and referred even in the homozygotes of the OTC defect to recurring migrainous symptomatology.

It is now clear that these rarities, and their exploration, have led us to realize that the heterozygous state of the same defect, and perhaps of other urea cycle defects, or of defects which can indirectly interfere with the function of this cycle and related activity, may also have clinical expression, and predominantly so in episodes of migraine and cyclical vomiting. The actual attacks themselves may be most closely relatable to the recurrent elevations of blood ammonia which build up in response to cumulative protein stress or other more specific dietary items like chocolate, apart from other stress of nondietary character.

The endpoint of its neurovascular effect is still likely to be the local vascular mechanisms encompassing the initial preheadache vasoconstrictive phase in brain and retina followed by regional arterial dilatation of temporal vessels, etc., which have long been advocated as the source of headache by the school of Wolff. I envisage this, therefore, as a specific self-protective shunt of cerebral flow. It is, of course, also possible that other inborn errors may be identified in which specific dietetic loading or other stress peculiar to them, could provoke excess of their correspondingly abnormal precursor or metabolite, to which the neurovascular and/or neurohumoral receptors of the migrainous reaction are equally susceptible. In such an intrinsically abnormal situation, the migraine reaction may yet prove to be a warning or defensive signal averting further intake of the offending dietetic substrate, while at the same time shutting off, or limiting, vascular entry of the harmful metabolite to highly sensitive retinal cells or other neuronal integrations. Severe lack of an end product could perhaps engender the same effect. "Exhalations" or "vapors" were indeed extolled by the ancients as the source of most clinical pathology, including migraine. Unwittingly, our ancestors were not so wrong in at last one family of disorders. Apart from its rare homozygous members, an intrinsic metabolic disorder leading to abnormal blood concentrations of ammonia at least underlies one form of migraine and cyclical vomiting.

The account we have unfolded illustrates how some light can be thrown upon our understanding of a very common disorder by the study of a rare one.

### Acknowledgments

For so much of the biochemical assistance and for so many of the other details of the work embodied in this paper, I owe a sincere debt of gratitude to Dr. Margaret Davidson, Marion Statter, Ph.D., V. G. Oberholzer, Ph.D., Shula Abzug-Horowitz, M.Sc., and Mrs. Mira Meshulam.

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# Scar Cancer of Lung Complicating Rheumatoid Lung Disease

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The pulmonary lesion of rheumatoid disease has been known to pathologists for many years (1, 2). Generally patients with rheumatoid lung disease suffer mostly from dyspnea on exertion and many eventually develop cor pulmonale. The syndrome may develop in aggravated form when associated with pneumoconiosis, as in Caplan's syndrome (3, 4).

The case described here is that of a 63-year-old woman with rheumatoid arthritis in whom a highly anaplastic cancer of the right lung gave rise to metastases of enormous size in a relatively short time. Autopsy revealed typical findings of rheumatoid lung disease with extensive honeycombing.

*Case Report:* A 63-year-old woman was admitted to the hospital because of abdominal pain in the right lower quadrant, increasing constipation, and weight loss during a period of 4 weeks. Previously she had been under treatment for arthritis of about 20 years' duration. During the past year she had been suffering from increasing pain so that Indocin® was substituted for salicylates. For three weeks she had pain which grew steadily worse in the right hip and thigh. During recent months she had persistent productive cough with yellow viscid sputum and a feeling of warmth with sweats, especially in the afternoons. There was no expectoration of blood. The patient gave a history of having been a heavy smoker.

Physical examination revealed a pale, chronically ill woman who had evidently lost some weight. A few rhonchi were audible at the lung bases. A systolic murmur was heard over the heart near the apex. Palpation of the abdomen disclosed an egg-size mass in the right lower quadrant. The hemoglobin was 10.5 gm percent, hematocrit, 34; WBC, 29,500; neutrophiles, 86; sedimentation rate 125 mm/hr (Westergren). The urine contained a trace of protein. The chest film revealed a large lobulated mass lesion located somewhat anteriorly just above the right hilus. The lesion was held to be highly suspicious for primary bronchogenic neoplasm although the possibility of metastasis was not ruled out. The lungs also exhibited some generalized interstitial fibrosis. X-ray examinations of the spine and pelvis showed osteoporosis of senile type and a healing fracture in the right inferior pubic ramus, possibily a pathologic fracture. Intravenous pyelogram revealed normal appearance of the kidneys and suggestive evidence of a pelvic mass extending toward the right side.

Following admission the patient had fever ranging between 98.8 and 101.6. She developed increasing tenderness over the mass in the right lower abdomen. Eventually a bulge appeared in the right inguinal region indicating a pointing abscess. Incision and drainage yielded about 1100 cc of foul-smelling greenish-yellow pus from which B. proteus was cultured. The site of drainage was found to communicate with a huge retroperitoneal abscess pocket extending downward into the pelvis and upward into the right lumbar gutter behind the cecum and descending colon.

For a period of ten days after the operation the patient felt remarkably improved, then developed pain in the right upper quadrant of the abdomen with shock tenderness over the liver. A mass now became palpable in this region and liver scan showed evidence of metastatic disease. The patient died two and one-half months after admission.

*Autopsy Findings:* The subject was an emaciated white woman of 63 years in moderate rigor mortis, weighing 90 pounds and measuring 5′3″ in height. Moderate edema

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was present in both lower extremities. The surgical incision in the right inguinal region measured 4.5 cm in length.

The surface of both lungs was stippled with anthracotic pigment. The right lung weighed 500 gm, the left 400 gm. A large tumor mass 5 × 4.5 × 4 cm occupied about one-third the substance of the right upper lobe in its upper anterior portion extending upward to within 3 cm of the apex. The tumor was sharply circumscribed above and below by a narrow capsule of compressed lung tissue. On section the tumor was milky white and finely granular. Just beneath the overlying pleura an area of dense anthracotic scar was encountered, measuring 3 × 2 × 1 cm, comprising the hilus of the tumor. An elliptical nodule of tumor tissue 2 × 1 × 0.5 cm protruded into the lumen of the left main bronchus almost to the level of the carina. The nodule was covered with a smooth, translucent layer of mucosa. Section through the nodule revealed it as part of an enormous mass of metastatic tumor, measuring 3.8 × 3.5 cm replacing the tracheobronchial lymph nodes and closely apposed to the Y of the tracheobronchial bifurcation. The tumor tissue was partly broken down and had a pale, brownish, spongy appearance on section.

The remainder of the lung tissue in both lungs was moderately indurated and poorly inflated. Cut sections revealed in many areas a tracery of anthracotic scar tissue in the form of a network enclosing minute cystic spaces.

Microscopically the tumor consisted of bulky polyhedral cells with abundant, finely granular cytoplasm and large, often multilobulated nuclei. The tumor exhibited a compact glandular organization with papillary proliferation of its cells into large irregular lumens. In places the tumor had a somewhat looser organization with tendency to form acinus-like structures not unlike a primitive alveolus. Many tumor cells had swollen and desquamated into the lumens of these structures. Much old and recent necrosis was present. Sections taken through the subpleural scar revealed dense fibrous tissue containing scattered rudimentary gland-like nests of tumor cells morphologically similar to the benign gland-like formations found in old lung scars.

Other parts of the lung exhibited multifocal interstitial fibrosis with secondary cystic emphysema and many foci of lymphocytic infiltration. In many areas interstitial fibrosis had advanced to the stage of "honey-comb lung" and the residual air spaces were lined with a continuous layer of cuboidal alveolar epithelial cells showing some atypism. The bronchi exhibited much hyalinization of basement membrane and mucous cell hyperplasia characteristic of chronic bronchitis.

The pericardium was free of exudate or adhesions. The heart was somewhat enlarged (360 gm) as a result of pronounced hypertrophy and dilatation of the right ventricle, which averaged 0.7 cm in thickness. The left ventricle was of ordinary thickness (1.4 cm). The endocardium of the valve and chambers was unremarkable. On section the myocardium appeared pale.

The coronary arteries were widely patent. Microscopically the branches of the major coronary arteries exhibited old intimal hyalin fibrosis and residual chronic inflammation. The left ventricle showed minimal subendocardial fibrosis. The mitral leaflet and mitral ring were slightly thickened and hyalinized.

The liver was enormously swollen with metastatic tumor. It weighed 4300 gm and bulged prominently toward the anterior abdominal wall displacing other viscera downward and to the left and elevated the right diaphragm to the level of the third rib. The tumor cells were highly anaplastic in appearance and lacked the glandular organization of the primary tumor in the lung. Mitoses were bizarre, bulky, and of enormous size. In some fields the majority of the tumor cells were in mitosis.

An enormous tumor metastasis 9 × 7 × 5 cm occupied the lower abdomen and pelvis. The mass compressed the terminal ileum and appendix and had invaded the appendix. Most of the mass was necrotic and filled with pus.

Tumor metastases were noted in the thyroid, kidneys and pancreas. The spleen exhibited pronounced hemosiderosis. The bone marrow was cellular.

Sections of the brain revealed minor degenerative changes in the cerebellum including the presence of small, rounded basophilic structures resembling calcified psammoma bodies and some degeneration of Purkinje cells.

*Anatomic Diagnoses:*

1. Highly anaplastic adenocarcinoma of right upper lobe of lung, apparently arising in scar tissue (alveolar cell carcinoma).
2. Metastatic adenocarcinoma of tracheobronchial, peripancreatic, and periaortic lymph nodes.
3. Metastatic adenocarcinoma of liver with marked hepatomegaly.
4. Metastatic adenocarcinoma of left lobe of thyroid gland, pancreas and right kidney.
5. Metastatic adenocarcinoma of ileocecal lymph nodes with extension to cecum and appendix.
6. Status after incision and drainage of retroperitoneal abscess arising in suppurating metastases of ileocecal lymph nodes.
7. Rheumatoid arthritis (clinical).
8. Extensive rheumatoid disease of lungs with secondary advanced fibrosis (honeycomb lung).
9. Cachexia and emaciation.

## Comment

A curious bias which equates all lung carcinomas with bronchogenic carcinoma pervades the thinking of many physicians. This was understandable when opinion prevailed that the only epithelium possessed by the lung was that lining bronchi and bronchioles. Alveoli were held to be bare of epithelium and lined instead with phagocytic cells of mesenchymal origin.

Current opinion now favors the thesis that the alveoli possess a continuous lining of epithelium. Alveolar epithelium is endowed with remarkable properties as organizer and protector of alveolar structure and function. It serves a scavenger function to cleanse the alveoli of foreign material by phagocytosis and provides the continuous secretion of pulmonary surfactant to permit the alveoli to enlarge freely during inspiration.

These properties of alveolar epithelium are largely suppressed within pulmonary scars. Surviving alveolar epithelial remnants persist within such scars as "gland-like formations" composed of low cuboidal epithelium. Pathologists for years have recognized these structures as forerunners of certain types of lung cancer ("scar cancer") (5), yet this knowledge has only recently begun to influence the thinking of clinicians (6).

My own experience with scar cancer of the lung extends back to over 35 years ago when I became associated with the Pulmonary Group of The Mount Sinai Hospital, New York, headed by the late Harry Wessler. The group included Coleman B. Rabin, the late Harold Neuhof, and others.

At that time the surgery of lung cancer was in its pioneer phase. Autopsy observations gave compelling proof that certain types of lung cancer clearly lent themselves to surgical excision with the promise of cure. These cancers were classed as "parenchymal carcinomas" because of their circumscribed growth and their location close to the pleural surface of the lung. When lung surgery came into its own it claimed many successes, a majority of which concerned parenchymal cancers.

With the increasing confidence and competence of thoracic surgeons many more such cancers were accumulated in laboratories of surgical pathology. In many instances the promise of cure by lobectomy or pneumonectomy was fulfilled. With wider experience it became increasingly evident that such favorable outlook for parenchymal cancer was far from universal. Many alveolar cell cancers were found to pursue a highly malignant course with widespread metastases and early death.

*Clinical Characteristics of Parenchymal Lung Cancer:* The clinical picture of parenchymal cancer is usually distinctive. Diagnosis in many instances comes about when routine x-ray screening of the chest is performed in asymptomatic persons. This arises from the circumstance that parenchymal lung cancers are often clinically silent because of failure to involve the major bronchi in their early stages so that the patient has few or no pulmonary symptoms. In a few cases the presenting symptom is chest pain, resulting from direct invasion of the chest wall. Productive cough with bloody sputum the hallmark of bronchogenic carcinoma, occurs late in the course of parenchymal carcinoma and generally corresponds to the presence of secondary necrosis and cavitation within the tumor.

The x-ray findings are often sufficiently characteristic to permit diagnosis without biopsy. This in an important consideration because of the fact that bronchocsopy is usually unrevealing of tumor unless metastases to regional nodes have eroded the adjacent bronchial wall. The tumor usually produces a large, dense filling defect in the lung, more or less rounded or slightly lobulated ("coin lesion"). In certain views a pleural dimple can be identified as an indentation on the surface of the mass.

When central necrosis occurs, the tumor may empty its liquefied contents through an eroded bronchus to be expectorated as bloody sputum. In such cases the x-ray appearance may suggest a tuberculous cavity or lung abscess. Generally the unusually thick wall of the cavity above the air-fluid interface betrays its tumor origin.

*Pathologic Characteristics of Scar Cancer of the Lung:* Commonly the telltale dimple in the pleura marks the location of the underlying tumor. Cut section through the dimple generally reveals dense scar tissue heavily impregnated with anthracotic pigment in witness to its advanced age contrasted with the relatively brief life span of the cancer. The scar often seems to constitute the hilus from which the tumor grows inward toward the deeper substance of the lung. At times the tumor fills and distends an entire lobe and invades the chest wall peripherally and the large bronchi and blood vessels at the root of the lung. Central necrosis often leads to cavitation of the tumor.

There is good reason to believe that many "bronchogenic carcinomas" of the lung are actually scar cancers of the lung which are misdiagnosed at bronchoscopy or autopsy because of the fact that the primary tumor in the lung periphery has dispatched metastases to the hilar lymph nodes. Tumor tissue in these nodes penetrates the adjacent bronchus to appear in its mucosa as an endobronchial growth. I have observed this sequence in several instances.

The histologic features of parenchymal scar cancers have confused many observers. A term commonly employed is "terminal bronchiolar cancer," largely because of the reluctance of many pathologists to acknowledge the existence of alveolar epithelium and of its potential for giving rise to carcinoma. While it is true that some scar cancers give rise to bronchiolar adenocarcinoma, as suggested by the presence of ciliated epithelium within the more mature parts of the tumor, the great majority of scar cancers are lacking in this feature.

The histologic features of parenchymal carcinoma of scar tissue origin are often sufficiently characteristic to permit ready classification instead of relegating it to such waste-bin categories as "poorly differentiated adenocarcinoma" or "giant cell carcinoma." The most revealing findings histologically are those which comprise features possessed by alveolar epithelium in contrast to bronchial or bronchiolar epithelium.

Classification of lung cancer as alveolar cell carcinoma is generally possible if the following features can be demonstrated:

1. The formation of gland-like units of large size with complex infoldings suggestive of giant uninflated fetal alveolar ducts.
2. The tendency of the tumor units to mimic fetal primary pulmonary lobules. The dichotomous branching of the tumor units is often demonstrated best in metastases of the tumor to certain tissues, especially the adrenal glands and vertebrae.
3. The discovery of densely hyalinized scar of obviously long duration within the "hilus" of the tumor, typically beneath the surface dimple in the pleura overlying the tumor.
4. The presence of atypical gland-like structures within the scar which are intermediate between "gland-like formations" of ordinary pulmonary scars and the anaplastic elements of florid tumor growth. This gradation, when identifiable, is convincing evidence of the relation of the tumor to the parent epithelial elements in the scar.
5. The cytologic features of the tumor cells, which are suggestive of alveolar epithelium. In general the cells are bulky and tend to be ovoid or pear-shaped with rounded, free borders facing the lumen. In cases of well-differentiated alveolar cell carcinoma the tumor cells desquamate freely into the lumen and often exhibit the foamy cytoplasm typical of ordinary alveolar phagocytes. The tumor cells in such instances stain strongly positive with the PAS technique, a characteristic of the alveolar phagocyte.

In other instances the alveolar cell carcinoma is composed of highly anaplastic tumor cells, including many giant and bizarre cell types in which the features of alveolar epithelium are difficult to identify.

Not infrequently scar cancer may evolve as epidermoid carcinoma, presumably the result of squamous metaplasia of tumor-forming epithelium.

The fact that patients with parenchymal lung cancer are often heavy users of tobacco, as in the case of bronchogenic carcinoma, perhaps reflects the fact that exogenous carcinogens are importantly concerned in the etiology of both types of lung cancer. The carcinogenic chemical may act directly at predisposed sites within bronchi or may be trapped within the crevices of scar tissue, where it exerts its mutagenic activity upon the alveolar epithelial remnants.

*Scar Cancer as a Specific Entity:* Scar cancer of the lung has its parallel in other organs, such as the liver, skin, breast, pancreas, and kidney. The example of epidermoid carcinoma arising on the basis of the scar of an ancient skin burn (Marjolin's ulcer) is well documented. The classical example of scar cancer is hepatoma arising on the basis of cirrhosis, especially postnecrotic cirrhosis or hemochromatosis. I have observed a number of instances of carcinoma of the pancreas arising in dense scars which presumably represent the site of ancient pancreatitis. Hypernephroid tumors of the kidney are often found to arise at the site of an ancient scar and multiple small premalignant hypernephroid tumors are not uncommon in the ischemic scars of kidneys damaged by arteriosclerosis.

Theoretically, any type of scar tissue in the lung may give rise eventually to scar cancer. Long duration of the scar appears to be important. My own experience suggests that a minimum of 13 years of apparent quiescence is required for scar cancer to develop on the basis of healed tuberculosis. In the typical case increasing density appears in the region of the old scar in successive chest films taken in routine follow-up. At first this finding suggests reactivation of tuberculosis. Eventually it proves to be superimposed scar cancer. This sequence underscores the importance of continued follow-up observation in quiescent tuberculosis.

Among the many causes of scar tissue formation in the lung pulmonary tuberculosis ranks foremost. Accordingly scar cancers of the lung are most commonly found in the apical region. Such cancers may constitute the syndrome once described as "Pancoast tumor" when the tumor invades the supraclavicular fossa and brachial plexus (7).

Silicosis, although a strong provocative of extensive pulmonary scarring, appears, paradoxically, to provide much less background for lung cancer than other types of scar-producing disorders. The paradox is difficult to explain except possibly on the basis of continued inflammatory activity about foci of silicosis, as reflected in the large numbers of plasma cells about these lesions. Accordingly silicotic foci fail to enter into the prolonged quiescence of other types of scar tissue, which appears to be a necessary condition for the evolution of a scar cancer.

Numerous instances of "scar cancer" of the lung, usually alveolar cell cancers, have come to light in cases of pulmonary fibrosis resulting from scleroderma (8, 9), the Hamman-Rich syndrome (10), asbestosis (11–14), and interstitial fibrosis of uncertain cause (15, 16).

In a related study of pulmonary changes in rheumatic heart disease (270 autopsied cases), I encountered 15 cases of primary lung cancer (more than 5 percent), among which 8 were classified as alveolar cell adenocarcinoma (17).

Search of the available medical literature has failed to uncover a case of scar cancer arising on the basis of rheumatoid lung disease.

## Summary

Clinical and pathological criteria exist for identifying certain lung cancers as originating in alveolar epithelial remnants within ancient pulmonary scars. Their tendency to remain localized justifies an aggressive surgical approach with hope of cure.

The existence of pulmonary scar tissue, regardless of cause, particularly in patients addicted to smoking, warrants careful follow-up observation in order to detect scar cancers in their earliest stages while opportunity exists for removal. Scar cancer arises often on the background of healed inactive tuberculosis but may occur in patients with extensive pneumoconiosis, particularly asbestosis, or pulmonary fibrosis complicating scleroderma, rheumatic heart disease, and other conditions. A case is described in which highly anaplastic alveolar cell adenocarcinoma was associated with pulmonary scarring secondary to rheumatoid lung disease.

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# Parenteral Clindamycin Therapy of Severe Infections

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Clindamycin, (7(S) chloro-7-deoxylincomycin), a new semi-synthetic antibiotic, has been shown to possess greater in vitro activity than the parent compound, lincomycin, against gram-negative anaerobes and gram-positive cocci, excluding the enterococcus (1). The clinical usefulness of this drug in the therapy of severe infections has recently been suggested (2–4). The present study describes our experience with the use of the parenteral form of clindamycin (clindamycin-2-phosphate) in the treatment of serious infections in a general hospital.

## Materials and Methods

Seriously ill patients admitted to The Mount Sinai Medical Center between December 1970 and February 1972 who were suspected of having infections due to gram-positive bacteria or gram-negative anaerobes were selected for parenteral clindamycin therapy. Prior to institution of therapy, appropriate aerobic and anaerobic cultures were obtained and processed by the diagnostic microbiology laboratory. Bacterial isolates were tested for sensitivity to clindamycin using 2 $\mu$g and 10 $\mu$g discs by a modified Kirby Bauer method (5) and by the standard two-fold tube dilution method. In most instances, besides the minimal inhibitory concentration (MIC), minimal bactericidal concentrations (MBC) of the drug for the isolate were also determined. Disc and two-tube dilution sensitivity tests (6) were also performed with erythromycin, penicillin-G, oxacillin, tetracycline, kanamycin, streptomycin, chloramphenicol, ampicillin, gentamicin, lincomycin, cephalothin and vancomycin. Blood samples were obtained prior to, during, and immediately following cessation of therapy for determinations of hematocrit, platelet count, white blood cell and differential counts, reticulocyte count, blood urea nitrogen (BUN), serum creatinine, electrolytes, alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), creatinine phosphokinase (CPK), and bilirubin. Routine urinalyses were also abtained.

Three to four hundred mg doses of clindamycin were administered intravenously or intramuscularly at either 4, 6, or 8 hour intervals for a dosage range of 1.2 to 2.4 gm/day, depending upon the nature and site of the infection. The duration of therapy was adapted to the clinical setting and ranged from 1 to 3 weeks.

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## Results

Clindamycin was administered parenterally to 28 seriously ill patients, 21 males and 7 females, whose ages ranged from 17 to 81 years. Twenty pathogenic organisms were isolated in 19 of the patients. Nine patients were presumed to have bacterial infections by their clinical presentation, response to therapy, and gram stain results, although cultures ultimately proved negative.

The infections treated with clindamycin are listed in Table I. Of the 28 patients, 6 had bacteremia—3 of pulmonary origin; 2 secondary to bacterial endocarditis; and 1 secondary to a perinephric abscess. The 22 nonbacteremic cases consisted of 12 instances of cellulitis or fascial plane infections; 1 each of pyogenic arthritis and osteomyelitis; and 8 serious respiratory tract infections, including 3 pneumonias, 2 lung abscesses, 1 empyema and 2 upper respiratory tract infections in compromised hosts.

The 20 pathogenic organisms recovered from clinical material in 19 patients consisted of 8 isolates of Staphylococcus aureus; 4 of Streptococcus pyogenes; 3 of Staphylococcus epidermidis; 2 of Bacteroides sp.; and 1 each of D. pneumoniae, non-grouped Streptococcus, and Actinomyces bovis (Table I). All of the isolates were inhibited by 2 $\mu$g clindamycin discs. The MIC of clindamycin in all cases was less than 0.38 $\mu$g/ml; for 7 of the isolates the MIC's were $<0.10$ $\mu$g/ml. All the strains of S. aureus were resistant to penicillin-G.

The clinical data and responses to therapy are summarized in Table II. Twenty-two patients recovered from their infection with clindamycin therapy; two patients (Patients 20 and 22) had partial responses; and 3 failed to respond to the drug (Patients 12, 25, and 28).

TABLE I
*Types of Infection and Bacterial Isolates*

| Infection | Number of cases | Number of isolaes | Organisms (No.) |
|---|---|---|---|
| Cellulitis | 12 | 9 | S. aureus (4)<br>S. epidermidis (2)<br>S. pyogenes (3) |
| Pharyngitis with otitis media | 1 | 0 | — |
| Arthritis | 1 | 1 | S. pyogenes |
| Osteomyelitis | 1 | 1 | S. epidermidis |
| Pneumonia | 3 | 1 | S. aureus |
| Bronchitis | 1 | 0 | — |
| Lung abscess | 2 | 1 | Actinomyces bovis |
| Empyema | 1 | 1 | Bacteroides sp. |
| Bacteremia | | | |
| Pneumonia | 3 | 3 | Bacteroides sp.; D. pneumonia; and Streptococcus (not grouped) |
| Perinephric abscess | 1 | 1 | S. aureus |
| Endocarditis | 2 | 2 | S. aureus (2) |
| Total | 28 | 20 | |

## TABLE II

*Clinical Data of Patients Treated with Parenteral Clindamycin*

| Patient No. | Age/Sex | Diagnosis | Isolate | 7-chlorolincomycin Therapy | Response | Side Effect |
|---|---|---|---|---|---|---|
| 1 | 71/M | Cellulitis of left leg and foot | — | 300mg IV q6h × 10 days | Rapid cure | — |
| 2 | 64/F | Cellulitis of vulva; diabetes mellitus; Felty's syndrome | — | 300mg IV q6h × 10 days | Rapid cure | — |
| 3 | 39/F | Cellulitis of left submandibular gland, floor of mouth and cheek | — | 300mg IV q6h × 11 days | Excellent clinical response; infection pointed and was drained by needle aspiration | — |
| 4 | 42/M | Pustules on feet bilaterally with lymphangitis and draining inguinal nodes | S. aureus; S. pyogenes | 300mg IV q6h × 10 days | Rapid cure | Masculopapular rash |
| 5 | 48/F | Cellulitis of left foot and leg following insect bite | S. aureus | 300mg IV q6h × 10 days | Rapid cure | — |
| 6 | 56/M | Cellulitis of left calf involving surgical wound | S. pyogenes | 300mg IM q8h × 10 days | Rapid cure | — |
| 7 | 55/F | Cellulitis of left foot and leg | S. pyogenes | 300mg IM q8h × 10 days | Rapid cure | — |
| 8 | 18/M | Postules and cellulitis of entire scalp | S. epidermidis | 300mg IM q6h × 13 days | Rapid cure | — |
| 9 | 55/M | Cellulitis of left wrist at IV site; acute myelogenous leukemia | S. aureus | 300 mg IV q8h × 5 days<br>300 mg po q6h × 5 days | Rapid cure | — |
| 10 | 19/M | Cellulitis and phlebitis of left forearm; drug addiction | S. aureus | 300 mg IM q8h × 10 days | Rapid cure | — |
| 11 | 43/M | Cellulitis and wound infection over acrylic prosthesis postcraniotomy | S. aureus | 300 mg IV q6h × 10 days | Wound was debrided, response was excellent | — |
| 12 | 59/M | Polycythemia vera; myeloid metaplasia; postsplenectomy; cellulitis right buttock | — | 300 mg IV q6h × 7 days | No response Infection resolved when drug was discontinued | — |
| 13 | 63/F | Pharyngitis and otitis media; acute myelogenous leukemia in relapse | — | 300 mg IV q6h × 3 days; 300 mg po q6h × 6 days | Rapid response | — |
| 14 | 28/F | Asthmatic bronchitis | — | 300 mg IM q8h × 7 days | Satisfactory response over 7 days | — |
| 15 | 17/M | Right middle lobe pneumonia; heroin addiction; hepatitis B | — | 300 mg IV q6h × 7 days | Rapid cure | — |
| 16 | 33/M | Right upper lobe pneumonia | — | 300 mg IV q6h × 2 days; 300 mg po q6h × 10 days | Rapid cure | — |
| 17 | 32/F | Diffuse necrotizing pneumonia after barbiturate ingestion | S. aureus | 300 mg IV q6h × 7 days; 300 mg po q6h × 7 days | Rapid cure | — |
| 18 | 42/M | Right lower lobe pneumonia and empyema | Bacteroides sp. | 300 mg IM q6h × 11 days | Chest tube drainage; excellent response | — |

TABLE II—*Continued*

| Patient No. | Age/Sex | Diagnosis | Isolate | 7-chlorolincomycin Therapy | Response | Side Effects |
|---|---|---|---|---|---|---|
| 19 | 42/M | Left lower lobe lung abscess; bronchiectasis | Multiple coliforms | 300 mg IV q6h × 12 days | Had bronchoscopy and lavage; excellent clinical response | Maculopapular rash |
| 20 | 74/M | Right upper lobe lung abscess | Actinomyces bovis | 300 mg IM q6h × 22 days | Sputum Sterilized; cavity remained | — |
| 21 | 56/M | Vertebral osteomyelitis and meningitis; diabetes mellitus | S. epidermidis | 400 mg IV q6h × 21 days; 300 mg po q6h × 3 mos. | Rapid response with eventual cure | — |
| 22 | 22/M | Bursitis and septic arthritis of right shoulder; cellulitis of right arm; cocaine addict | S. pyogenes | 300 mg IM q8h × 6 days; 300 mg po q6h × 4 days | Initial response good but relapsed | — |
| 23 | 36/M | Bacteremia; perinephritic abscess; heroin addict | S. aureus | 400 mg IV q6h × 19 days | Surgical drainage; rapid response | — |
| 24 | 72/M | Bacteremia; bilateral pneumonia; diabetes mellitus with Retoacidosis; renal insufficiency | D. pneumoniae | 300 mg IV q6h × 10 days | Rapid cure | — |
| 25 | 81/M | Bacteremia; bronchopneumonia; chronic obstructive lung disease | Streptococcus sp. (not grouped) | 300 mg IV q6h × 2 days | Died | — |
| 26 | 78/M | Bacteremia; right lower lobe pneumonia; organic mental syndrome | Bacteroides sp. | 300 mg IV q6h × 6 days | Rapid cure | Maculopapular rash; hepatic dysfunction |
| 27 | 34/M | Acute bacterial endocarditis; abscess of right foot; methedrin addict | S. aureus | 300 mg IV q6h × 15.5 days | Excellent response but signed out of hospital | — |
| 28 | 47/M | Bacterial endocarditis; rheumatic heart disease with mitral insufficiency; chronic renal failure with polycystic kidneys | S. aureus | 300 mg IM q24h × 9 days | Fever and positive blood cultures persisted | — |

## *A. Cellulitis* (*Patients 1–12*)

Eleven of the 12 patients with cellulitis or wound infection treated with clindamycin exhibited an excellent clinical response within 36 hours.

Illustrative Case: Vulvovaginal cellultis. A.C. (Patient 2), a 64-year-old diabetic woman with Felty's syndrome and leukopenia developed severe cellulitis involving the entire external female genitalia associated with fever. Repeated cultures of aspirated material were negative. Despite the empiric use of multiple antibiotics, including cephalothin, kanamycin and tetracycline, no improvement was observed.
Intravenous clindamycin was begun in a dose of 1.2 gm/day, and a good response occurred within 36 hours, associated with defervescence and eventual complete resolution of the infection after 10 days of therapy.

One patient in this group (Patient 12) with polycythemia vera and cellulitis failed to respond to this drug. No organism was cultured from the lesion or the blood stream.

### *B. Respiratory Infections*

Clindamycin was effective in the treatment of 7 of 8 patients with serious nonbacteremic respiratory infections (Patients 13–19), and effected a partial response in the 8th case (Patient 20). These included 2 severe upper respiratory tract infections in compromised hosts: a case of severe otitis media and exudative pharyngitis in a 63-year-old woman with acute myelogenous leukemia (Patient 13), and a case of acute bronchitis in an asthmatic patient on chronic corticosteroid therapy (Patient 14). In both of these cases, x-rays and cultures were negative; however, gram stain of the sputum in Patient 14 revealed predominantly gram-positive lancet-shaped diplococci. A clinical response within 48 hours after institution of clindamycin was noted.

Three patients with extensive pneumonia were treated (Patients 15–17). No pathogens were isolated from Patients 15 and 16. However, in both of these cases, chest x-rays revealed lobar consolidation and gram stains of the sputum showed many gram-positive diplococci and neutrophiles. Serial M. pneumoniae complement fixation titres were negative. Both patients responded with lysis of fever within 36 hours after institution of clindamycin therapy, and gradual clearing of the chest x-rays.

Illustrative Case: Staphylococcal pneumonia. A.O., (Patient 17), a 32-year-old woman, presented with barbiturate overdose and respiratory depression that required 72 hours of assisted ventilation. While on the respirator, sputum cultures consistently were positive for S. aureus, coagulase-positive, sensitive to clindamycin. Following discontinuation of respirator therapy, the patient developed patchy pulmonary infiltrates, spiking fevers, progressively increasing dyspnea, purulent sputum, and leukocytosis. S. aureus was again isolated from the sputum. Intravenous clinadamycin was initiated in dosage of 1.2 mg/day. Rapid clinical improvement ensued within 36 hours, with eventual resolution of the pneumonia.

One patient with empyema was treated with clindamycin with excellent response. This case is detailed to illustrate the use of clindamycin in a patient with severe anaerobic infection.

Illustrative Case: Empyema secondary to Bacteroides sp. The patient (Patient 18) presented with a 2-week history of right upper quadrant abdominal pain and right-sided pleuritic chest pain, associated with fever and anorexia. Chest x-ray revealed a right pleural effusion. Aspiration yielded thick, green, foul-smelling fluid from which Bacteroides sp. were isolated. The MIC of clindamycin for this organism was 0.09% $\mu$g/ml. The patient was treated with clindamycin and chest tube drainage for 11 days with complete recovery.

One of 2 patients with lung abscesses treated with clindamycin responded. Patient 19 was a 42-year-old man with clinical and chest x-ray findings of putrid lung abscess. Cultures of sputum yielded multiple gram-negative bacilli of the Enterobacteriaceae family, all resistant to clindamycin. On the presumption, however, that the pathogens were most likely anaerobic organisms (7) a course of parenteral clindamycin was initiated with excellent clinical and radiological response. The other patient with a lung abscess (Patient 20) was suspected of having an underlying bronchongenic carcinoma. Although he became afebrile, and sputum cultures became negative, the cavity persisted.

### *C. Skeletal Infections*

Two patients with skeletal infections were treated with clindamycin. One (Patient 21) developed meningitis and vertebral osteomyelitis due to S. epidermidis following myelography. Following a partial response to intravenous oxacillin therapy and recovery of this organism from a bone biopsy 4 weeks later, he was treated with parenteral and then oral clindamycin for an extended period. This was accompanied by radiological and clinical evidence of cure of the infection.

A cocaine addict (Patient 22) with septic arthritis and cellulitis of the arm partially responded to therapy but signed out of the hospital after 8 days of therapy.

### *D. Bacteremic Infections*

Three of 6 patients with bacteremic infections responded to therapy.

There were 3 cases of bacteremia associated with pulmonary infections (Table II). Two patients had bacterial endocarditis due to S. aureus. Patient 27, a drug addict admitted with fever of 105° F, the murmur of tricuspid insufficiency, and an abscess on the foot, had blood and abscess cultures which yielded S. aureus, coagulase-positive. The other patient (Patient 28) with endocarditis, a 47-year-old man with rheumatic mitral insufficiency, maintained on chronic hemodialysis via an arterial-venous fistula, was admitted with fever, chills, and chest pain. When S. aureus was recovered from 4 sets of blood cultures, clindamycin was administered (300 mg/day). Neither clinical response nor sterilization of the blood stream was seen following 9 days of therapy at which time oxacillin was begun with prompt response.

### *E. Side Effects*

The drug was well tolerated in the patients. There were no instances of phlebitis, pain at injection sites, renal or hematologic toxicity. Two patients complained of a bitter taste during intravenous infusions. Three patients (Patients 4, 19, 26) developed maculopapular rashes. In 2 instances (Patients 4 and 19), several other drugs were also being administered concomitantly. In one of these patients (Patient 19) all drugs were discontinued and clindamycin was readministered with no recurrence of the rash. A more severe reaction merits detailed description:

Illustrative Case: A.W. (Patient 25), following twelve 300 mg doses of intravenous clindamycin, developed a maculopapular rash with laboratory evidence of hepatic dysfunction as evidenced by rises in serum alkaline phosphatase from 85 to 150 international micro units (I$\mu$U), and SGOT and SGPT from normal values to 600 and 500 I$\mu$U respectively. The patient was receiving no other medications at that time, although immediately preceding the institution of clindamycin, he had received penicillin, cephalothin, and gentamicin. Clindamycin was discontinued on the 6th day of therapy. The patient's clinical condition did not permit liver biopsy; however, all liver function tests returned to normal levels within a few days following cessation of therapy. The rash resolved during this time.

## Discussion

Clindamycin proved to be an efficacious antimicrobial agent for the treatment of a variety of clinical infections due to gram-positive cocci and Bacteroides species.

Eleven of the 12 patients with cellulitis and fascial plane infections responded well to the drug. Only one treatment failure of 11 bacteremic and nonbacteremic respiratory infections was encountered and this occurred in a patient who was moribund on admission (Patient 25). An additional treatment failure occurred in a patient maintained with chronic hemodialysis (Patient 27) in whom the dosage of clindamycin was presumably too low to maintain effective blood levels. The favorable though limited experience with the use of clindamycin in the treatment of serious infections due to Bacteroides sp. (Patients 18 and 26) supports the view emerging in the literature of the effectiveness of this durg against anaerobic organisms (4, 8). An in vitro study of 75 strains of Bacteroides sp. by Chow and Guze (8) revealed 98.5% of strains to be sensitive to chloramphenicol (mean MIC, 6 μg/ml) and 96% to be sensitive to clindamycin (mean MIC, 0.8 μg/ml). Clindamycin phosphate, alone or in combination with an aminoglycoside, was administered parenterally to 30 patients with infections due to non-spore-forming anaerobic bacteria and 26 responded to the therapy. In vitro susceptibilities correlated well with clinical response. Romig et al (3) cured five patients with bacterial endocarditis with clindamycin, including 1 due to Bacteroides fragilis.

We did not encounter organisms resistant to clindamycin in our study. Meyers et al (9) found that all 43 strains of S. aureus tested were inhibited by <0.12 μg/ml of clindamycin. Geddes et al (10) reported that 475 out of 500 S. aureus and 78 out of 100 H. influenzae isolates were sensitive to 2 μg clindamycin discs. The MIC of clindamycin for 32 strains of S. aureus was <0.8 μg/ml and 2 strains were inhibited by 3.2 μg/ml. Streptococci and pneumonococci are usually exquisitely sensitive to clindamycin (1, 9) although resistant Group A Streptocoicci have been reported (11).

No local side effects were noted in our patients when clindamycin was given intramuscularly or intravenously. In several patients the duration of therapy exceeded three weeks without adverse effects. Three patients, however, developed maculopapular rashes and one of these also had evidence of reversible hepatic dysfunction. Fass and Saslaw (12) also noted only transient transaminase elevation in a significant number of their patients treated with intramuscular clindamycin. Swenson and his coworkers (4) reported only 2 drug reactions in 32 patients treated with clindamycin; one patient developed a maculopapular rash and another developed transient thrombocytopenia.

## Conclusion

Twenty-two of 28 patients who received parenteral clindamycin were cured of their infections. Three patients failed to be cured on this therapy and the remaining 3 could not be evaluated. Rashes were observed in 3 patients and 1 developed liver enzyme abnormalities. Initial evaluation suggested that clindamycin will be an effective agent for the treatment of infections secondary to gram-positive cocci (other than enterococci) and gram-negative anaerobic bacilli.

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# Rashes and Arthropathy in Viral Hepatitis

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Individual cases of infectious hepatitis HA (old terminology, IH) and serum hepatitis HB (old terminology, SH) are clinically indistinguishable. HA behaves as do many other acute primary viral infectious diseases, with a fairly regular incubation period, an early rise in the level of IgM, the appearance in convalescence of specific humoral antibody which in the form of human immune serum globulin can prevent or modify IH in exposed susceptible persons. Recovery is generally without hepatic sequelae and without a long-term carrier state. On the other hand, HB may be associated with a variety of serious chronic hepatic disorders, a carrier state of significant duration and diverse extrahepatic manifestations.

A recent publication dealing with immune-complex arthritis in viral hepatitis stated that arthralgias may occur in the prodromal period both in HB and in HA (1). However, the two references cited in support of arthralgia in IH do not sustain this. The first describes three patients who are likely candidates for HB, a married couple who shared equipment for intravenous use of drugs and a physician who had recently returned from one year of work in East Africa doing clinical and epidemiologic studies of patients with various liver diseases (2). The other paper cited in support (3) also concerns three patients: two were house physicians who developed rash and arthritis prior to the appearance of icteric hepatitis; the third patient was a 34-year-old black woman seen in 1965 with rash and arthritis followed by mild hepatitis; when recalled in 1970 her serum test for HB Ag was negative. The first house-physician's serum was HB Ag negative when tested seven weeks after onset of joint symptoms, the second house-physician's serum was negative when first tested a year after illness. The conclusion that rash and arthralgia resulted because of HA in those six patients is very tenuous; two were intravenous drug users, three were physicians whose occupational exposure to both HA and HB is recognized. Especially at the intervals cited, a negative test for HB Ag cannot be regarded as excluding SH.

In an attempt to clarify the question, it was decided first to review the reported findings in a unifocal outbreak of HA and one of HB which met the following criteria: 1) The episode responsible for the ensuing epidemic of IH or SH must have been precisely described; and 2) The clinical description of the patients must have been adequately recorded. Two reports meet these criteria.

A unifocal epidemic of unquestionable HB occurred in 1942 among healthy young males injected with human plasma on a single day. (4) in contrast, a striking epidemic of unquestionable HA occurred in 1969 among healthy young males infected orally with contaminated water, also on a single day (5).

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### *The Unifocal SH Epidemic:*

266 British soldiers of a single troop were injected on one day with mumps convalescent pooled plasma; their average age was 19, their general health and fitness good. The 11 blood donors had recovered from mumps and were members of the same troop. The ensuing attack rate of clinical hepatitis with jaundice and biliuria was 44%. The incubation period was 59–94 days. We reported "the hepatitis was mild and except for the common skin manifestations and arthralgias, resembled epidemic infective hepatitis as seen among children and young adults in Britain" (6).

When skin and joint manifestations occur in HB, the signs appear from several days to several weeks before hepatitis becomes apparent. The first men involved were admitted to the skin department of a British military hospital; others were initially seen because of arthritis. Significant arthralgia was recorded in 25% of cases and skin rashes (urticaria, angioneurotic edema, papular and erythematous reactions) in 42% of those who subsequently developed jaundice. A number of the inoculees developed rashes and arthralgia but no clinical jaundice subsequently, suggesting that such findings may also occur in anicteric HB. (Liver enzyme tests such as SGOT were not available.)

Of the 266 inoculated men, 11 gave a history of "catarrhal jaundice" in childhood, presumably HA. Of these 11, 8 nevertheless developed jaundice following injection of the mumps convalescent plasma. It was evident that there was no cross protection between the two forms of hepatitis.

### *The Unifocal HA Epidemic:*

A college football squad of 97 healthy males consumed contaminated water on the practice field. An elegant epidemiologic study revealed that the responsible incident occurred on 29 August 1972. Ninety of the 97 individuals at risk developed hepatitis; 36 of these were asymptomatic, but were found to have SGPT levels in excess of 100 units. The outbreak began in the last week of September and reached its height early in October, displaying the typically short incubation period of HA. The squad was under health surveillance for illness and athletic injury. A tabulation of the signs and symptoms of the resulting cases of hepatitis (icteric and nonicteric) indicated that none had developed arthralgia, arthritis, or rashes. Early and serial serum specimens on all persons were negative for HB Ag and HB Ag antibody (7).

Numerous reports were reviewed of point source and time-limited outbreaks of hepatitis characterized by short incubation and associated with the ingestion of contaminated water, milk or foods. Outbreaks well described clinically and epidemiologically arising in resort hotels or camps are exemplified by such reports as (8–12). These outbreaks could be presumed to be due to HA virus. The absence of any reference to skin rashes or arthralgia is noteworthy.

Some epidemics of HB have had a high incidence of rashes and arthralgia, while others have not. The differences may reflect an inherent characteristic of the strain of HB virus concerned. Observations reported from a U.S. naval station in 1945 suggest this possibility (13). Inactivated influenza vaccine was administered subcutaneously to officers and enlisted persons. A "control group" received 1.0 ml of distilled water containing 0.03 ml of lyophilized pooled human plasma and 272 cases of hepatitis were subsequently identified. The authors reported "Certain symptoms of an allergic nature, namely hives, arthralgia and rash, were common early symptoms in this epidemic" (arthralgia, 37.9%; hives, 32.5%). The authors further noted (their Table III) that such findings were equally common in the plasma-inoculated and in 30 of their close personal contacts who later developed hepatitis. This was the earliest recorded evidence that secondary cases could arise in uninoculated individuals from personal contact with SH patients. It also suggested that the incidence of rashes and arthropathy was a characteristic of the particular strain of SH virus.

In contrast, the administration of yellow fever vaccine which was associated with 51,337 recorded cases of HB in United States' troops in 1942 had a low incidence of rashes and arthropathy. "It was reported at the time of the survey that at the hospital at Camp Mason three patients had shown urticaria two or three days before the jaundice appeared, and later it was learned at Letterman General Hospital there were about 20 patients in whom urticaria had appeared before the jaundice" (14). It is unlikely that a high incidence of such findings as was observed in the HB epidemic mentioned above would go unnoticed. It would thus appear that the incidence of rash and arthralgia as a pre-icteric finding in HB will vary with different epidemics.

Epidemiologic and clinical studies in Sweden suggest that HB is the chief cause of track-finders' hepatitis. (15) In a report of 320 track-finders hospitalized between December 1957 to May 1961, only 11% were reported as having arthralgia in the prodromal period. (16) In contrast is the observation in 80 cases of icteric HB in hospital personnel working in a Swedish hemodialysis unit. (17) Arthralgia occurred in 55% of the 80 personnel, always in the prodromal period of HB. The influence of strain differences in HB virus appears to be the explanation for the different incidence of arthralgia in the two Swedish groups reported.

## Discussion

Why should rashes and joint symptoms occur in HB and not in HA? That the two forms of viral hepatitis are fundamentally different is clear. The natural history of IH is that of other primary acute virus infections. On the other hand evidence of the association of HB Ag and HB virus in several varieties of progressive hepatic disease as well as in vasculitis and other extrahepatic manifestations such as rashes and arthritis suggest a more complicated process in which the role of circulating immune complexes may be an essential component in pathogenesis (18–20). Immunoelectron microscopy observations of Almeida "suggest certain similarities between HB Ag hepatitis and serum sickness, a condition where the severity also seems to depend on the balance between antigen and antibody" (21).

The recent publication by Popper and Mackay (27) on the relation between HB Ag and autoimmune hepatitis is of interest. It is proposed that the HB Ag "consists of a very small amount of RNA-enzyme complex (virion) with an amount of host protein far in excess of the protein coat of most other viruses. These host proteins include various preexisting structures of the liver cell." As little as 0.03 ml of infected human plasma is sufficient to produce HB disease. The enormous amount of HB Ag in the circulation suggests that antigen excess is the usual situation in the earlier stages of disease when serum-sickness-like rashes and arthropathy may be seen.

Why should the incidence of extrahepatic manifestations such as rashes, arthritis, and vasculitis vary from one outbreak of HB to another? It is reasonable to hypothesize that this reflects an inherent property of the strain of HBV involved. It is likely that there are pathobiologic differences in strains within the two major subtypes (*d* and *y*) of HBV. The *d* and *y* subtypes have been shown to breed true in circumscribed outbreaks of each in HB outbreaks in Holland (23) and in the United States (24).

Antigenic subtype specificities have now been successfully detected by radioimmunoassay in serums which were negative by immunoprecipitation (25).

## Summary

An epidemic of serum hepatitis (HB) resulted from the injection of mumps convalescent plasma on a single day. Forty-two percent of patients developed rashes and 25% arthropathy in the prodromal period.

An epidemic of infectious hepatitis (HA) resulted from drinking fecally contaminated water on a single day. None of the patients developed rashes or arthropathy. Other reported point source epidemics of IH were not associated with prodromal rashes and arthropathy.

Variations in the frequency of rashes and arthropathy in different outbreaks of HB may be explained by the existence of recently demonstrated distinct antigenic subtypes which breed true.

Patients with viral hepatitis giving a history of prodromal rashes and/or arthralgia should be suspected of having HB.

## Addendum

A sophisticated laboratory epidemiologic and clinical study of the subtypes of HB Ag indicates that subtypes breed true and that clinical features may vary with the subtype. This study was time and place limited, i.e., 1969 to 1972 in Copenhagen.*

It is recognized with a number of viral diseases that strains belonging to the same serotype may evoke varying clinical consequences. If so for HBV, it suggests that the high incidence of skin and joint findings in two of the epidemics discussed above (References 6 and 13) reflects a property of the viral strain rather than a host response.

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* Subtypes of Australia Antigen among Patients and Healthy Carriers in Copenhagen. Jens O. Nielsen, M.D., George L. LeBouvier, M.D., and the Copenhagen Hepatitis Acute Program. New England Journal of Medicine, 288:1257–1261, June 14, 1973.

The occurrence of these symptoms is accompanied by marked depression of serum hemolytic complement and C4 component (Reference 18). This raises the specter of delayed immune-complex disorders of extrahepatic localization such as polyarteritis, thrombophlebitis, and arthritis. Long-term follow-up of HBV patients whose initial episode of hepatitis was accompanied by skin and joint symptoms may prove very useful.

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# Bronchial Fistula Complicating Subphrenic Abscess

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Bronchial fistula complicating subphrenic abscess was first described by Cauderay (1) in 1902. Seven years later, Piquand (2) was able to report 890 collected cases of subphrenic abscess, 112 of which were associated with bronchial communications. In the collective review of subphrenic abscess reported by Ochsner and DeBakey (3) in 1938, the incidence of bronchial fistula in over 3600 cases was ten percent. Since that time, case reports and small series of cases of this complication have been reported, and the incidence of fistula is mentioned in some of the reported series of subphrenic abscesses as varying between one and 17 percent (4–7).

In a recent review of 45 patients with subphrenic abscess treated at The Mount Sinai Hospital, there were six patients who either presented with or developed a bronchial fistula. Three fistulae were associated with right-sided abscess, and three with left-sided abscess. Two of the fistulae followed perforation of the colon secondary to ulcerative colitis, and three followed gastric surgery. In one case the etiology of the abscess remains obscure.

## Case Reports

M.C., a 27-year-old man with ulcerative colitis, was admitted with six months of left chest pain and fever and five weeks of foul sputum. Roentgenograms demonstrated a communication between the splenic flexure and the left lower lobe of the lung. A methylene blue enema produced blue sputum within minutes. Ileostomy was performed with complete amelioration of symptoms, and this was followed by colectomy. Following colectomy, the left subphrenic space drained for one year but then finally stopped, and he went on to subsequent proctectomy and complete recovery.

C.R., a 38-year-old woman, was admitted with diffuse peritonitis following a tube enterostomy for perforated ulcerative colitis performed at another hospital. She underwent multiple operations for revision of her ileostomy and drainage of wound and intraabdominal abscesses. The last site of infection was a left subphrenic abscess with a bronchial fistula presenting with cough and foul sputum. The left subphrenic space was drained by the abdominal route, and the pulmonary problem cleared completely. She subsequently had a staged colectomy performed without difficulty.

D.B., a 53-year-old man, was admitted to the hospital for the first time with a history of right upper abdominal pain and fever of four months' duration. He was thought to have a liver abscess and was treated intensively with antibiotics. His fever and symptoms subsided and he was discharged only to be readmitted three weeks later with fever, cough, and foul sputum. Immediate laparotomy was performed with drainage of a right subphrenic abscess. A defect was noted in the right diaphragm, and postoperative irrigation of the cavity produced a cough and expectoration of the irrigating fluid. He went on to complete recovery.

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A.C., a 73-year-old man, underwent emergency gastric resection for bleeding gastric ulcer. Postoperatively he ran fever with roentgenographic evidence of a high right diaphragm. Twenty days after resection he was explored for right subphrenic abscess, but no abscess was found. Following this procedure he developed cough and foul sputum. Within a week, he was reexplored, this time via a posterior approach, and a right subphrenic abscess was found and drained. After this procedure, injection of the abscess cavity demonstrated a fistula to the right lower lobe which subsequently closed.

P.M., a 50-year-old man, underwent gastrectomy for bleeding at another institution. This was followed by wound dehiscence and a persistent duodenal fistula. Two months postoperatively a right subphrenic abscess was drained via a posterior approach. The drainage tract closed; fever recurred and was now accompanied by cough and foul sputum. After three months of recurring fever, pain, and purulent sputum, he was transferred to The Mount Sinai Hospital. On the operating table during intubation, a large amount of creamy, purulent material appeared from the right tracheobronchial tree. At laparotomy, an abscess high over the dome of the right diaphragm was drained of a large amount of pus which was similar to that in the bronchus. A defect was noted in the diaphragm. The duodenal fistula was found to be closed; it did not communicate with the abscess. Cough and sputum stopped immediately postoperatively and he had an uncomplicated recovery.

R.M., a 28-year-old man, was transferred to The Mount Sinai Hospital for treatment of a persistent left subphrenic abscess. The patient had sustained a perforated duodenal ulcer ten weeks previously which was closed at another hospital. The postoperative course was complicated by a left subphrenic abscess which was drained both anteriorly and posteriorly. He continued to run fever, developed a cough productive of thick, purulent sputum, and was then transferred. Bronchography revealed a communication between the left lower lobe and the left subphrenic space. He required several operative procedures including splenectomy before adequate drainage could be achieved. Immediately after the splenectomy, his condition improved rapidly, and he recovered.

## Discussion

Bronchial fistula occurs as a result of perforation of a subphrenic abscess through the diaphragm into the overlying adherent lung. If the lung is not adherent to the diaphragm, empyema will follow the perforation. While fistula formation is usually a late complication in the course of subphrenic infection, in Case 4 (A.C.) it was diagnosed within three and one-half weeks after the original surgery.

Five of the six patients presented here were males, the age ranged from 27 to 73 years. Three fistulae involved the right lung, and three the left. Two of the fistulae followed colonic perforation secondary to ulcerative colitis, three followed emergency surgery for gastric and duodenal ulcers, and in one patient, the etiology of the subphrenic infection was never determined. There were no mortalities in this group developing bronchial fistulae.

The diagnosis of bronchial fistula must be suspected when any patient who may have subphrenic infection develops cough and then purulent sputum. The diagnosis becomes fairly definite when the sputum is similar in appearance and culture to the pus in the abscess cavity. Injection of the abscess cavity with contrast media or bronchography will demonstrate the fistula in a high percentage of cases.

The treatment of the fistula is the treatment of the subphrenic abscess. As soon as adequate drainage is instituted, cough and sputum stop. In no case in this series has it been necessary either to drain or resect pulmonary tissue. Despite the bronchial infection and communication of the bronchus with the subphrenic space, no patient has developed any late pulmonary sequelae. This is in sharp contradistinction to a broncho-biliary fistula such as develops following perforation of a liver abscess into a bronchus. Here, the development of bronchiectasis is the rule because of the destructive effect of bile upon the bronchial mucosa.

In four patients the communication closed promptly following adequate drainage of the abscess. In one patient with ulcerative colitis, foul sputum stopped after ileostomy; and in one patient splenectomy was performed in order to provide adequate drainage of the subphrenic space. Thoracic drainage was not necessary, and there were no late pulmonary sequelae.

## Summary

Six cases of bronchial fistula complicating subphrenic abscess are presented. Treatment consists of adequate drainage of the abscess below the diaphragm. There were no mortalities or late pulmonary sequelae in this series.

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# The Role of Splenectomy in the Management of Left Subphrenic Abscess

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Despite the advent of antibiotics, subphrenic abscess still remains a complication associated with considerable morbidity and mortality. Because of problems in early diagnosis, compounded by the masking effect of antibiotics and pitfalls in effecting adequate drainage, the left-sided collection has continued to be the more difficult to manage and its prognosis poorer (1–4).

We recently reported six cases of bronchial fistula complicating subphrenic abscess (5). One of these patients with a left-sided abscess ran a hectic course, uninfluenced by multiple surgical procedures, until splenectomy was performed. A review of the literature discloses no other similar case report. It was therefore felt that this case should be presented in detail to assess the role of the spleen and the performance of splenectomy in the management of this disease.

## Case Report

J.M., a 28-year-old white man, was transferred to The Mount Sinai Hospital for treatment of a persistent left subphrenic abscess. The patient had sustained a perforated duodenal ulcer ten weeks previously which had been sutured at another hospital. The postoperative course was complicated by a left subphrenic abscess which was drained two weeks later via a posterior approach through the bed of the resected left twelfth rib.

The patient continued to run a febrile course with a gradual defervescence. He was finally discharged after seven weeks in the hospital only to be readmitted one week later with chills and fever. A second attempt was made to effect complete drainage of the left subphrenic space, this time through an anterior subcostal incision. Once again the patient continued a febrile course, now complicated by a persistent cough productive of thick, yellowish-green sputum. Because of progressive deterioration during the next three weeks he was transferred to The Mount Sinai Hospital for further treatment.

On admission the patient was desperately ill. The temperature was 102 degrees Fahrenheit and the pulse rate 120 per minute. There was clinical evidence of a left pleural effusion. Both the left subcostal and left flank wounds were exquisitely tender. The former was open and draining large amounts of greenish purulent material. There was a small draining sinus in the flank wound. Roentgenograms of the chest revealed a left pleural effusion with air-fluid levels in the left lower lung field (Fig. 1).

It was felt that the patient had an incompletely drained left subphrenic abscess with a perforation through the diaphragm into the left lower lobe. Additional x-rays appeared to localize the abscess anteriorly. On the day following admission, the left subphrenic space was explored through the subcostal incision with the evacuation of only minimal amounts of purulent material. The wound was packed open. The patient continued to run a low-grade fever with a persistent productive cough, the sputum being similar in appearance

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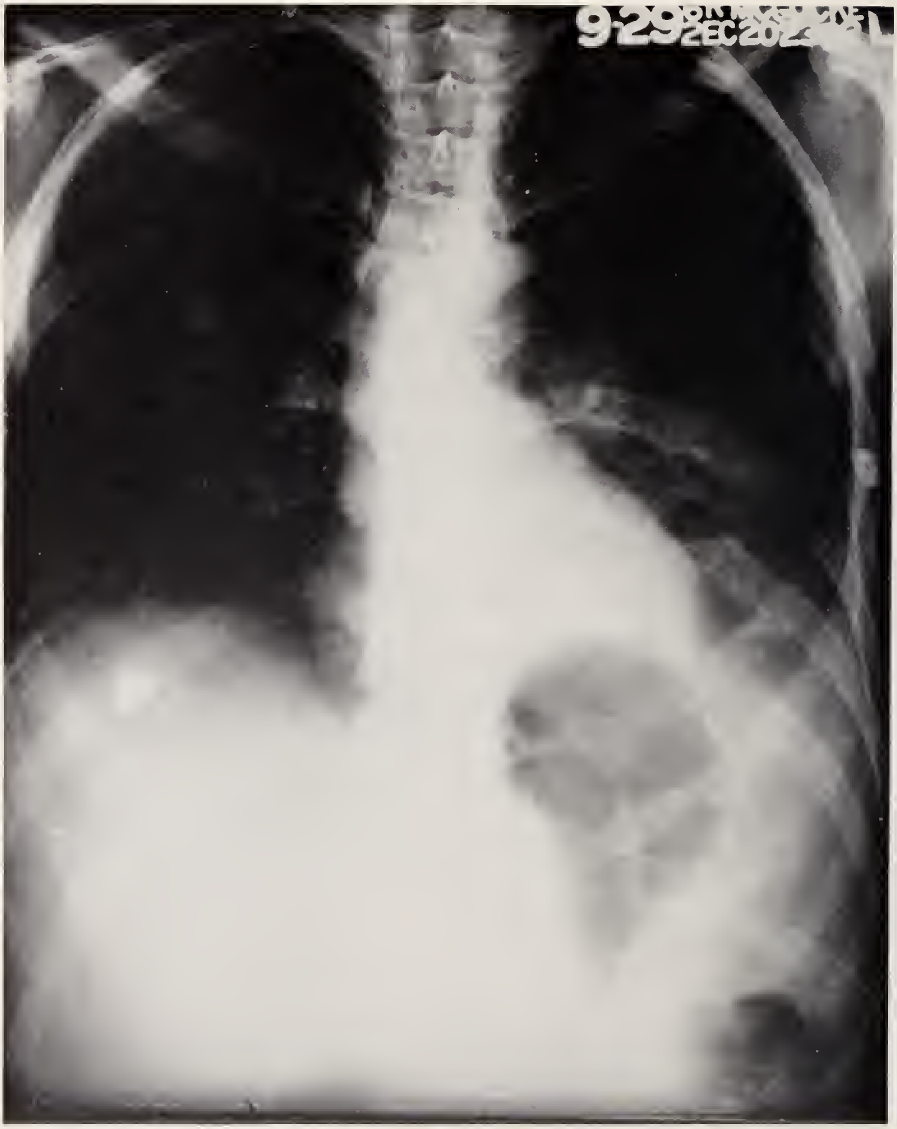

FIG. 1. Admission chest film demonstrates a left pleural effusion with air-fluid levels in the left lower lung field just lateral to the cardiac silhouette.

to the drainage from the abscess cavity. Both the pus from the subphrenic space and the sputum cultured B. pyocyaneus and Staphylococcus aureus. A selective left lower lobe bronchogram demonstrated a communication between the left lower lobe bronchus and the left subphrenic space without evidence of endobronchial disease (Fig. 2). Subsequent x-rays of the abdomen revealed retention of contrast media within the suppurative collection both above and below the diaphragm (Fig. 3).

During the next month, the left subphrenic space was explored on two subsequent occasions, both through the anterior and posterior incisions. In addition, because of the

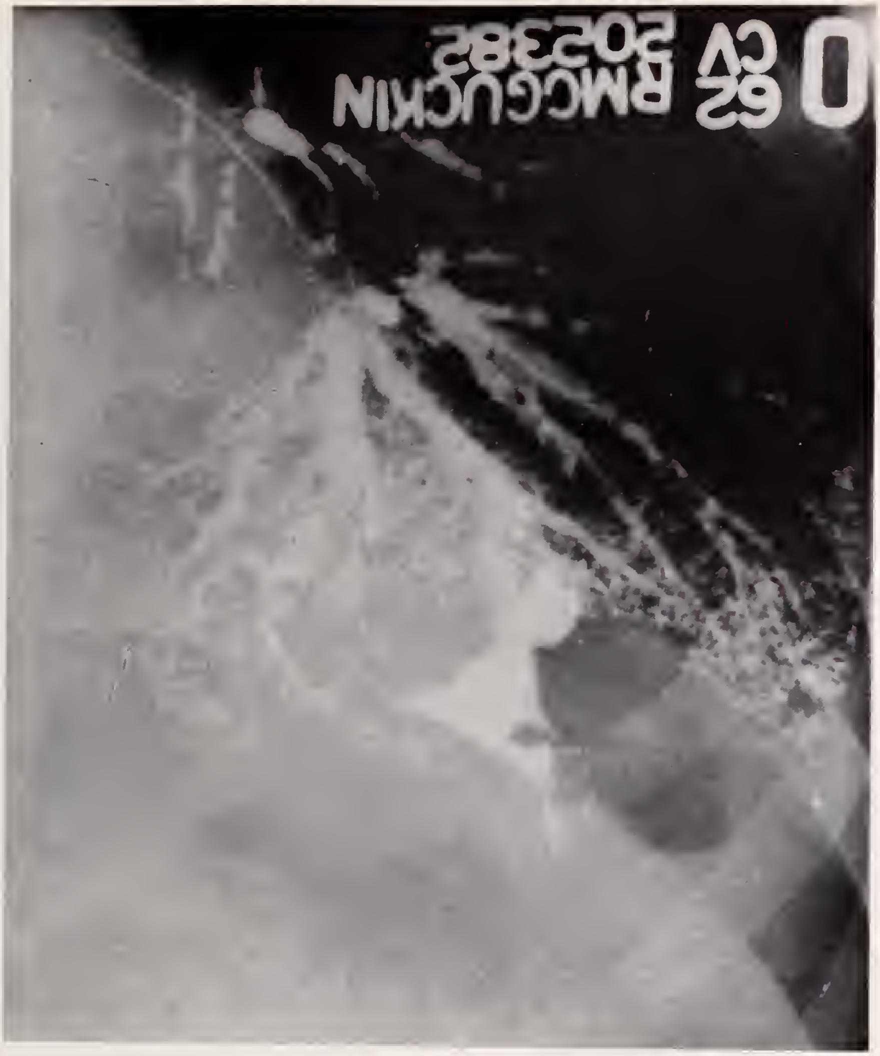

FIG. 2. A selective left lower lobe bronchogram demonstrates extravasation of dye beyond the confines of the bronchial tree below the diaphragm.

possibility of intrahepatic abscesses or an unrecognized intraabdominal abscess being responsible for the patient's protracted course, he underwent a formal laparotomy with negative findings. Despite the many surgical procedures and full course of antibiotics including penicillin, staphcillin, neomycin, and polymycin, the patient continued his downhill course, aggravated by a B. pyocyaneus bacteremia.

Because of the conviction that residual infection existed between the stomach and the left parietes, the decision was made to reexplore and open all tissue planes and spaces below the left diaphragm. This was done five weeks after admission. The spleen was found to be enlarged and soft. It was removed, thereby laying open the whole subphrenic area. Following removal of the large septic spleen, a defect could be visualized in the left diaphragm

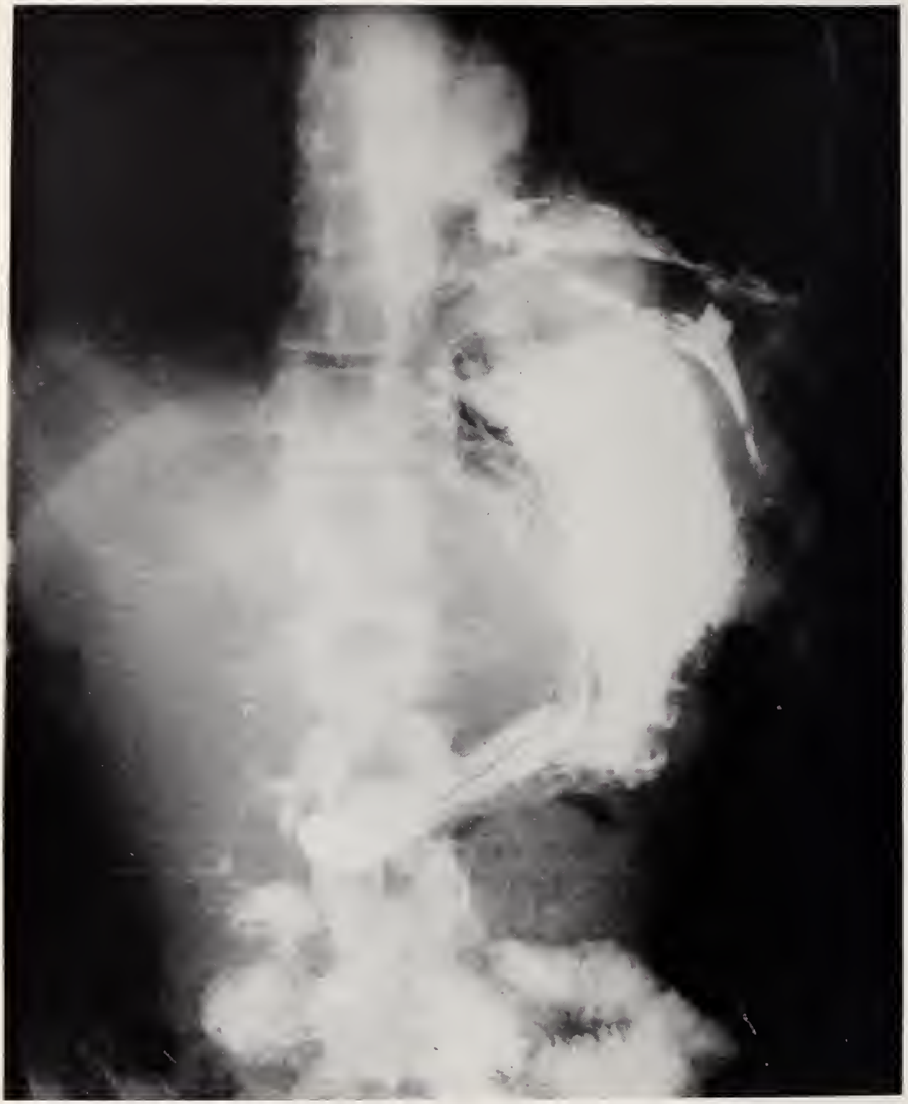

FIG. 3. An upper G.I. Series reveals no extravasation of barium beyond the confines of the stomach or duodenum. Retained Lipiodol® was present both supradiaphragmatically as well as in the subphrenic space lateral to the stomach.

with a direct bronchial communication. The wound was again packed widely. On pathologic examination the spleen contained neither macro- nor microabscesses.

The subsequent course was dramatic. His temperature fell to normal immediately after surgery. His appetite improved at once, and he began to gain weight rapidly. The fistula between the subphrenic space and the bronchus closed with disappearance of his cough and purulent sputum. The wound became clean and began to granulate, and he was finally discharged two months after admission.

His improvement continued, the wound healed completely, and follow-up roentgenograms have demonstrated clearing of the chest (Fig. 4).

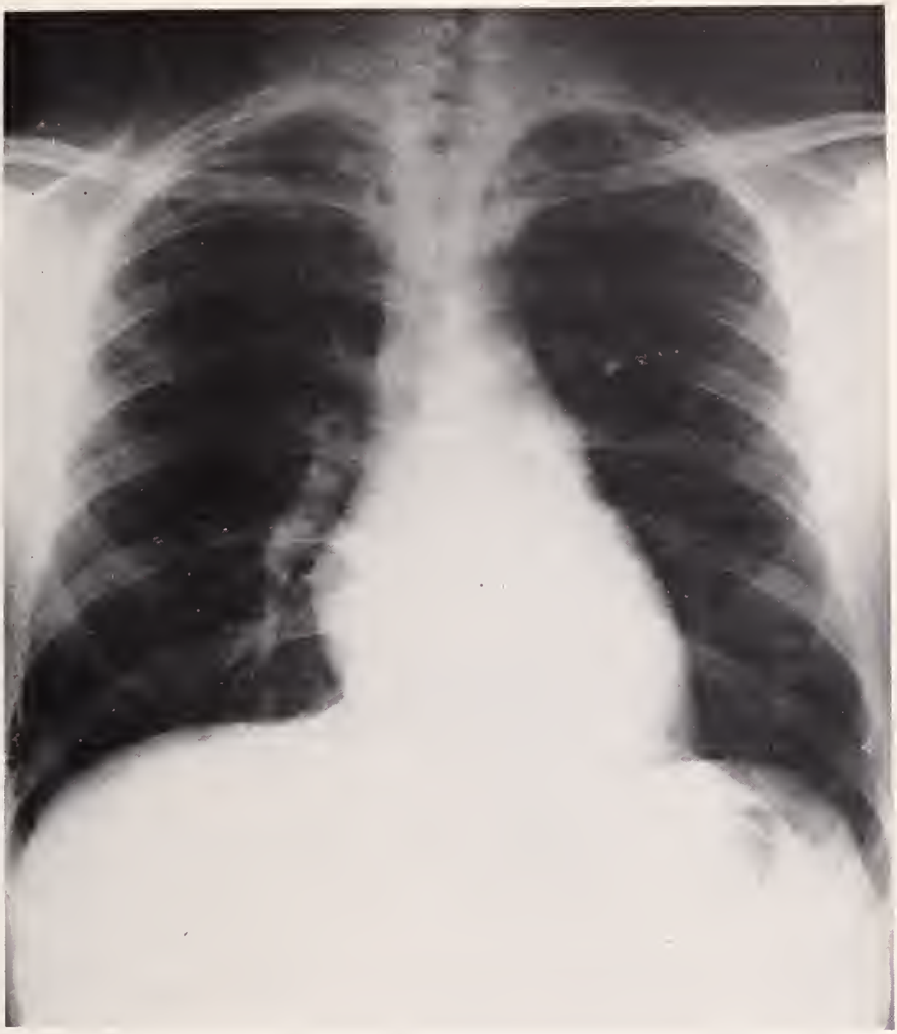

FIG. 4. Posterior anterior chest film six months postsplenectomy reveals an essentially normal chest. The patient was gainfully employed and completely asymptomatic.

## Discussion

Because of a fundamental anatomic difference between the right and left subphrenic spaces, the pathology, clinical features, and course of a subphrenic abscess vary considerably depending upon the side involved. On the right, the liver not only occupies the entire space, but is relatively immobile. Consequently, the suppurative process expands within a relatively closed space, and elevation of the hemidiaphragm is an early manifestation, facilitating prompt diagnosis of a right subphrenic abscess (3, 6–9).

By way of contrast, the left subphrenic space is only partially occupied by a relatively mobile left lobe of the liver. The other organs of the left subphrenic space, particularly the stomach and colon, are even more mobile. Since these viscera bordering a left-sided subphrenic collection are readily displaced by an enlarging abscess, the suppurative process is able to expand downward. Consequently the hemidiaphragm may not be elevated, making early diagnosis more difficult. Of even greater pathologic significance is the fact that the left-sided abscesses are apt to be multiloculated. This is often due to the interposition of the stomach and/or colon into the expanding abscess cavity (3, 6, 8).

Furthermore, as was the situation in the case presented, the spleen may also be responsible for the loculation with consequent ineffectual drainage of the partitioned space. Although alluded to in the literature (3), this must be very uncommon, since no similar case has been reported. In this instance, the infectious process continued for as long as the spleen remained as a barrier; once splenectomy was performed, free drainage of the loculated cavity was effected. Complete drainage was followed by a dramatic resolution of the morbid process.

Although not directly pertinent to the case presented, associated thrombosis of the splenic veins may cause infarction of part of the organ. Rabin (3) described two cases of left subphrenic abscess that ran a protracted course with fragments of splenic tissue being extruded from the wound periodically. Only after splenectomy, with removal of the residual splenic tissue was the process brought under control.

Bronchial fistula occurs as a result of perforation of a subphrenic abscess through the diaphragm into the lung. If the lung is not adherent to the diaphragm, empyema will follow the perforation. While fistula formation is usually a late complication in the course of subphrenic infection, it may become apparent within three to four weeks after the original surgery. The treatment of the fistula is the treatment of the abscess. Once adequate drainage is instituted, the fistula will close spontaneously, and late pulmonary complications are most rare.

The extraperitoneal route has long been advocated as the technique of choice for drainage of subphrenic abscess, either through the bed of the resected twelfth rib or via a subcostal approach (10, 11). Neuhof and Schlossman (8) described and advocated a one-stage transthoracic, transpleural procedure for selected cases. We would agree with Halasz (12), however, that the transabdominal approach is more effective in enabling the surgeon to find and deal with multiple abscesses, or extensive loculations within a single abscess. With proper care, the likelihood of peritonitis developing postoperatively is rare. The exposure obtained by formal laparotomy will help to identify a situation comparable to that found in the present case where the spleen acts almost as a foreign body within an abscess cavity and where its presence prevents adequate drainage. Should splenectomy be necessary, it can be performed more safely through a laparotomy incision. Laparotomy does not militate against dependent drainage. Indeed it facilitates the proper placement of dependent drains either posteriorly or in the flanks.

## Summary

A case is reported of a left subphrenic abscess with bronchial fistula which was cured only after performance of splenectomy. A review of the literature reveals no similar reports. When interposed between the loculations of a subphrenic abscess, the spleen may act as a barrier to adequate drainage.

Laparotomy is advocated as the approach of choice in the surgical management of subphrenic abscess.

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# Puerto Rican Perspectives on Mental Illness

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## *Cultural Bias in Symptom Reporting*

The doctor's new dilemma with people of different cultures mirrors the epidemiologist's growing uneasiness with straight symptom checklists as indicative of the true prevalence or incidence of many diseases.

This is the first of three articles reviewing some Puerto Rican beliefs and behavior surrounding health and illness. Whether by default or design most often the patient decides from his own experience and perspectives when to present himself to and what to report to the doctor—and what he will do with the medical advice he receives. What the doctor selectively hears is in turn filtered through the cultural perspectives of the physician and others whose professional lives and relationships form the structure of a complex medical center.

In a similar way what the epidemiologist reads in his computer print-out is filtered through the process of selective reporting of respondents and culturally influenced questions designed by the investigator (1).

The more engulfed people are in their own structure, the less they see of its richness and intricacy—and its pervasive influence on their own perceptions and behavior. The "givens" within the structure of a medical center and those embedded in the Puerto Rican culture predict the most frequent areas of confusion and misunderstanding about Puerto Rican patients. The contrast between basic assumptions of the two cultures also suggests that surveys based on symptom reporting might overstate the comparative prevalence of psychiatric illness among Puerto Ricans (2). Differences in perspectives may at times also lead the Puerto Rican patient and his family into special difficulties in relations with psychiatric services.

Our attitudes about time, our valuation of privacy and quiet, our confidence in the trained expert, our faith in the capacity of medicine eventually to solve all manner of problems, our esteem for the inquiring mind, the "good question," and search for natural causes, and our social definitions of illness work together and are constantly reinforced in action in large medical centers. It is different in the Puerto Rican's structure.

The large hospital couldn't function without two basic perspectives about time. The first is a belief that time is valuable and to be employed, scheduled, and saved, but not wasted, "killed," or lost. The second is commitment to an orderly sequence and future time that is subject to influence through the way we spend our minutes today.

But the Puerto Ricans may wonder why the clock rather than the satisfaction of an exchange determines when to say good bye. They do not see why the time, rather than their feelings, indicates when to see a friend or when to seek help. "How do *I* know whether I will feel like coming to the clinic on Tuesday next week?" The Puerto Rican who believes in destiny is unlikely to have had

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much successful experience with long-range planning. Past and future run together in the world of spirits. Things can happen to thwart planned actions because of interference from the spirits. "The bus left me," rather than "I was too late to catch the bus" (3).

The Puerto Rican's life is structured, but it moves to a rhythm different from that we associate with our structure. People stop by to chat and stay all afternoon. Someone is in trouble and needs help *now*, and many friends and relatives gather, dropping what they might otherwise have done, and none knows what the future will bring. Friends or relatives go with the person in trouble to see a spiritualist at the time when the person is upset. One woman, recently moved from Puerto Rico, decried the lack of easy access to help from friends in the metropolis. "In La Esmeralda you can bum a nickel from anyone. But with these people, the more they have the more they want. It's everything for themselves. You can't imagine how unpleasant it is (to have no one to confide in). In La Esmeralda you can tell everybody about it and that sort of eases your heart" (4).

A walk-in clinic or the emergency room is more consistent with this pattern of easy, personal access to help "on demand"; phoning for an appointment two weeks in advance or being put on a waiting list is not.

Summer in Spanish Harlem and all year in San Juan rocks with blaring radios—in crowded apartments, in *bodegas* and bars, in the hands of young *machos* as they stroll along the streets. Noise, laughter, outdoor card tables, domino games, heated arguments, and gossip can be assaulting to the ears of people used to "Hospital, Quiet" signs. Hushed corridors and "No Visitors" signs can be equally jarring and ominous to one who grew up in *El Barrio.*

Each culture has what Edward Hall refers to as its own definition of "body space"; the distance felt appropriate before privacy is invaded (5). A room to oneself and no visitors may be treasured by some, but abhorred by others. One Puerto Rican woman told Oscar Lewis:

> Moving here to the housing project was practically the social worker's idea. She insisted and insisted . . . This place isn't like La Esmeralda, you know, where there's so much going on. Here, you never see any movement on the street, not one little domino or card game or anything. The place is dead. People act as if they're angry or in mourning. Either they don't know how to live or they are afraid to. It's true what the proverb says: "May God deliver me from quiet places; I can defend myself in the wild ones" (6).

We have faith in training, in technical competence, and in science, and even suspect that personal relationships or too much closeness can get in the way of the ability of the doctor to help. The Puerto Rican has faith in personal relationships, and wonders whether a stranger can be trusted to care, or could know enough about him to help. He is not so convinced of the benefits of training, not so confident nor so knowing about medical science (7). In an ambiguous situation, he looks for *signs* to tell him whether the expert knows how to help, and cares. About a psychiatrist, "How could he help me? He asked *me* what is wrong?" "The doctor didn't even shake my hand when I came in."

Mrs. Ketty Rey, a social worker who ran a service for young mothers in the South Bronx, reports that she learned not to try to take a social history at first meeting. "They are afraid and won't tell the truth at first. Later when they trust, they will tell" (8).

If disappointed in his search for symbols of reassurance, of concern, as he comprehends them, the Puerto Rican patient is a likely dropout from therapy.

Our own environment encourages us to ask questions, to search for natural causes and to challenge existing patterns and beliefs. "That is a good question," "We'll do research on it," "insight" into reasonable sequence are rewarded in our structure.

We expect patients to be prepared to give accurate information about natural events in response to direct questions and to ask for an explanation from us when they don't understand. This is relatively easy for patients who as children were encouraged to question and to search for answers—the kind of children who are products of homes likely to produce scholars and researchers (9).

But the structure of Puerto Rican relationships from family outward discourages questioning. Children must show respect to parents and not question. The good wife should be demure and unquestioning. Otherwise she would diminish her husband's authority. Outside the family and particularly with strangers, asking questions implies loss of face; "I would seem stupid," or it might indicate disrespect of authority. The Puerto Rican, even when he does not understand medical instructions and explanations, may raise no questions.

One of our psychiatric patients when she was called to make an appointment for a home visit, said "Yes, yes." Later at the visit, when she became comfortable, she confessed that she had not understood what was going to happen and had "worried all afternoon" about what she had agreed to. She said this often happened when she talked to a doctor.

A frequent source of guarded response from the Puerto Rican is pride and the fear of losing face. The average education, the average life expectancy among Puerto Ricans living here is higher than on the Island (10). Their families, as those of other immigrants, are generally more ambitious for change, better life, and better jobs than the people of their own social class who stay at home. Against such aspirations and the fact that they could manage the move, they may be particularly sensitive to not being treated with their culturally valued *respeto* (11). They seem susceptible to perceiving slights to their *dignidad* and resent judgmental challenges to their way of life and their values. Concern about their dignity may dispose them to say "yes" whether or not they agree or understand, and to give what they think is the socially acceptable answer. Guarded responses to questions from strangers can generate misunderstanding on both sides of the patient-physician relationship.

Transplanted from a balmy island where life is conditioned by diffuse and personal ties, the immigrant must try to find roots in New York's concrete, steel, stone, glass and impersonal relationships of business, bureaucracy and the professions. In this context stereotyped and bland responses may not reflect the personality and feeling, but rather the fact of being scared by the big hospital, afraid of disapproval, or fearful of seeming foolish.

Our medical culture is imbued with the conviction that a wide range of discomforts, disabilities and complaints are appropriately brought to a doctor's attention. Prolonged sleeplessness, to say nothing of hearing voices and seeing visions, should impel one to see a psychiatrist. But the Puerto Rican's social environment may encourage him toward accepting visions and voices—even toward reporting them to someone he trusts and who values *facultades*, or the ability to communicate with the spirit world in a trance-like state. In *espiritismo*, practitioners who may be gypsies or mediums, claim *facultades* as their "license" for providing advice. In *santeria*, the more structured system of practice, position in the hierarchy is closely related to attribution of *facultades* (12). At the top, a *babalow*, always a male, is followed by a *presidente*, or head medium, then the *santeros* and following them the novices who are "developing" their *facultades* and learning the rituals, and last, "the believers." *Facultades* come from the "gifted one's" special, protective Catholic saints with African names, the *protecciones* (13).

*Santeria* is practiced in a basement or storefront, the *centro*, or in house gatherings. *Santeros*, dressed in white or wearing an *ide* (beaded bracelet) and beaded necklaces can frequently be seen in our elevators on their way to minister to hospital patients.

We see behavioral disturbances as symptoms of illness, mild to severe, to be treated, not judged. The Puerto Rican seems more accepting of a wider range if idiosyncratic behavior in the people he knows, and to make exceptions for friends with "nervousness" or "asthma" or a variety of somatic disorders. However, he makes a sharp distinction between being "just nervous" and being *loco*. "To become a *loco* is to be bad, dangerous, evil. It means losing virtually all socially valued attributes" (14). Within the belief context of many Puerto Ricans, to go to a state mental hospital as a *loco*, to have shock therapy, to suffer memory loss following ECT can be the first step in assuming the feared, all-encompassing, dominant and enduring social role in a world where people know a lot about each other and remember. To circumvent the sting of his role, the sick person and his family differentiate between the possibility and the actuality of being *loco* and stress nervousness and somatic complaints that they hope the doctor *can* cure, or they turn to spiritualism where the trouble lies not within the individual or his family, but out in the world of spirits, and where hope for improvement is high (15).

The Puerto Rican's first year in New York is characterized by high rates of hospitalization for all manner of illness (16). In trouble, Puerto Ricans turn first to friends, relatives and spiritualists, and they tend to be sicker when they finally turn to the medical profession. They settle where they know people and patterns; shop in small neighborhood groceries where they can know and be known, though they pay more there (17). They get their jobs and their apartments through friends and relatives and consequently are clustered in certain work places, and crowded in housing. Small storefront churches thrive in their communities. So do botanicas that sell herbs, potions, Florida water, ointments, and incense prescribed by spiritualists. Mount Sinai Hospital's Community Medicine survey of our neighborhood located 24 botanicas between 103rd and 115th Streets, from Park to Second Avenue (18). Some botanicas are so busy that they have introduced the New York custom of giving out numbers for people who are waiting for their "prescriptions" or turn to buy (19). Besides these, some hardware stores have special sections of herbs, etc., at the back, as do shops listed in the phone-book yellow pages under "Religious Goods."

Within this fabric of the Puerto Rican's life, the spiritualist is convincing because she or her group demonstrates ability, concern, and authority by telling and predicting. She elicits information by a series of assertions from the general, which are most sure to be correct, to the more particular, as she responds to cues from the individual and group. "Something is troubling you . . . ," ". . . someone close." The person in trouble responds, unfolding his problem. The spiritualist's chances of making the right predictions and generating trust and faith in outcome are good, and enhanced by intimate relationships with neighborhood patterns and people and gossip. The spiritualist summarizes what is wrong and confidently says what must be done next (20). One of our psychiatric patients reported, "The spiritualist did most of the talking . . . she really cared . . . I felt better when I saw her."

The Puerto Rican community supports a wide and viable network of spiritualists, some specializing, some consulting with each other. A recent survey in New York found that 73% of the Puerto Rican outpatients in a mental health clinic reported visits to spiritualists. Overlap between spiritualism and Catholicism, or spiritualism and Protestantism, is a norm more than an exception, excluding Jehovah's Witnesses and the Pentecostal sects which have some of the social characteristics of spiritualist *centros*. Determination of the religious affiliation of a Puerto Rican by no means excludes belief in spirits (21).

Dan Wakefield quoted one Puerto Rican as saying, "If a man says he doesn't believe in spirits, it means you haven't talked to him long enough" (22). While this is an overstatement, some Puerto Rican patients who deny belief in spirits later are discovered to be visiting spiritualists. Dr. Carol Koplan at Mount Sinai Hospital reported, "It was only after I had worked with the patient (Jenny) for more than a month that I learned she was seeing a spiritualist. The social worker had discovered this from her husband" (23). In turn, the social worker, who worked closely with this patient and her family reported:

> The patient's mother denied any belief in spiritualism . . . [but said] the spiritualist makes Jenny "feel good" and "answers her questions." She can predict the future, she knows what happened to people in the past, and she talks in different voices in a trance-like manner. The mother said that the only time she has seen Jenny happy lately is when she returns from the spiritualist. The patient's spiritualist has actually been a long-time friend of the family and both the patient and her step-sister insist that the mother is a staunch believer in spiritualism. According to her daughters, the spiritualist had pre-

> vented their mother from "going insane four years ago." The spiritualist determined that the mother had a bad spell put on her and broke the spell. (24)

As in this family, the tendency to tell the physician "I do not believe, but *she* does" is not uncommon.

One psychiatrist in San Juan reported that he learned of relatives bringing in the patient so the doctor could calm him enough so they could then take the patient to a "genuine" therapist—a spiritualist medium (25). Some mediums do apparently send their clients who seem to be getting out of control to psychiatric clinics. Alice Bolocan, an anthropologist, and Ellen Smith, a social worker, visited the spiritualist who was seeing our patient, Jenny. The spiritualist had called in a fellow spiritualist as a "consultant." Both spiritualists talked openly about psychiatric patients they had seen. They explained they had made progress with their "patients" in spite of the interference from "evil spirits" and stressed dislike for shock treatment because "it impairs the brain," and fear of medication because "it interferes with a person's ability to communicate with the spirit world." They also criticized psychiatrists for not encouraging "belief in God and prayer" in their work with patients (26).

Outpatients at one psychiatric clinic commonly referred to psychotherapy as *pases*—symbolic gestures performed by mediums in order to cure. In 80 percent of the Rogler and Hollingshead sample of San Juan families, at least one spouse admitted belief in Spiritualism. Nearly half the families performed a variety of spiritualistic seances at home (27).

One Puerto Rican respondent explained his perception of what psychiatry might be:

> Mediums understand things that the doctor-psychiatrist does not, that is if the doctor is not a spiritualist . . . When the doctor does not know about spiritualistic matters, he should consult with a spiritualist, and in this way they could come to an agreement. Spiritualists would treat the spiritualistic part of the problem and thereby rid the individual of possible evil spirits. Psychiatrists could treat the nervous system, if this were affected. There would be much success then. (28)

The anthropologist Hsu claims that Americans tend to accept magic when it is cloaked in the scientific method (29). Computerized astrological charts may be an example. In contrast, the Puerto Rican may see science as more believable when it is presented with an aura of magic or ritual, as with the air of therapeutic mystery that surrounds vitamins, penicillin, syringe and needle which some Puerto Ricans associate with the good things the physician can do (30). A Washington Heights survey found Puerto Ricans the "least informed" about medically accepted knowledge of disease. Puerto Ricans also scored highest on "skepticism of medical care" (31).

Rogler and Hollingshead report that some Puerto Ricans stereotype psychiatrists as uncaring, or even punitive authorities who sit in judgment of them and categorize them into slots which the Islander believes are reprehensible (32).

Through seeing a medium, the person in trouble may continue to receive group support from the people he knows and needs. He does not become an outcast. Indeed, he is often accompanied by a circle of concerned family and friends on his visit to a medium.

In the framework of the Puerto Rican's life, the spiritualistic interpretations of the symptoms of the mentally ill are simple, credible in his belief system, and given in a setting free of the stigma that many Puerto Ricans associate with psychiatric treatment, the psychiatric hospital, and particularly the state hospital or insane asylum. To become a *loco* is to be viewed as having lost virtually all socially valued attributes (33). Once a person is defined as *loco* he will be treated as such in the foreseeable future. "Look at the *loco*. We make him jump like a goat" (34).

The spiritualist as believer and participant in the Puerto Rican culture may even turn affliction into a sign of grace by announcing to the sick man, his friends, and his family that the patient is endowed with special psychic faculties, *facultades*, that can offer him prestige in his group.

The possible frequent association between assignment of the powers of *facultades* and trance-like states, visions and voices may contribute to tendencies of some Puerto Ricans to believe they experience the symptoms. But more surely such a setting may incline people to admit to these symptoms more readily than would people of a culture where the same phenomena are viewed with distaste and alarm.

Surveys of mental health and illness do show relatively high rates of symptom impairment among Puerto Ricans. In view of the stress-producing possibilities of migration, and statistical associations between illness and migration, one cannot dismiss evidence of symptomatology as only an artifact of cultural expression. The extreme of cultural relativity would lead to the untenable stand that if everyone in a society has broken bones, then a broken bone is healthy.

In the area of mental health and illness there is perhaps special risk of taking the statistical norm to mean "normality" or health. In cross-cultural studies, for example, it is sometimes claimed that what American psychiatrists would identify as mental illness might be considered health in another culture. Yet Leighton and his colleagues found that the African people whom psychiatrists identified as pathological were most often also identified by their own tribesmen as in need of help (35). There is some relationship in any society between socially disapproved behavior and the assignment of the "mental illness" designation, but this by no means suggests that the two are synonymous (36).

However the way symptoms are reported can mask the actual prevalence of a disorder when two cultures are compared, and more important for the clinician, the culture does have large implications for the style of symptom reporting and the basic assumptions about cause and effect that are a part of the patient's life.

In a series of 131 patients admitted to the Lincoln Hospital after attempting suicide, Trautman found that the most characteristic feature of the suicidal act in the 93 Puerto Rican patients was its suddenness and its impulsive character (37). Puerto Rican suicide rate is higher for the young male than is the US rate. Opler reports remarkable patterned cultural variations in the style of behavior among schizophrenics in a mental hospital (38). Weinstein details patterned differences in the content of delusions and hallucinations produced by people of different cultures (39).

Zborowski described striking patterned variation in the way patients of three ethnic groups responded to pain even when observed in the same clinical setting and with essentially the same diagnosis (40). Zola studied the selective process of what the patient brings to the doctor by comparing 18 female and 19 male patients, diagnostically matched, all new admissions to the Eye, Ear, Nose and Throat Clinics of Massachusetts General Hospital (41). Among the patterned differences: "even when Italians had a diagnosed eye or ear disorder, they did not locate their chief complaints there, nor did they focus their future concern on these locations." Also they "presented significantly more symptoms in significantly more bodily locations and noted significantly more types of bodily dysfunctions." The Irish more often than the Italians denied pain as a feature of their disorder. For example, two women with diagnosed presbyopia and hyperopia were each asked "What seems to be the trouble?" The Irish patient said "I can't seem to thread a needle or read a paper." The Italian: "I have a constant headache and my eyes seem to get all red and burny." When the interviewer asked "Anything else?" the Irish patient said, "No, I can't recall any." The Italian patient said, "No, just that it lasts all day long and I even wake up with it sometimes." Zola concluded that Italian and Irish ways of communicating illness reflect major values and preferred ways of handling problems within the culture itself (42).

At the present state of knowledge we can no longer ignore the consequences of the impact of social structure and of cultural definitions on results of prevalence studies (43). Nor can we interpret accurately the patient's statements about his health and illness and his practices that relate to health without knowing something of the context from which he speaks (44).

The health professional's decisions about when to intervene in illness and what therapies to use should be made with knowledge of the patient's perceptions and his life away from the hospital. This becomes particularly significant for the mentally ill Puerto Rican patient when medical words or medical decisions can set in motion unintended patterns of response to the patient. Family and friends, and even years later, responses of strangers may be influenced by their perception of the meaning behind the medical designation or recommendation.

Care need be taken so that in attempts to help, the physician does not unwittingly rip apart the fabric of the patient's social life, or without meaning to, return him to his society with a stigma that he may never be able to overcome.

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# Small-Cell Dysplasia and In Situ Carcinoma of Mammary Ducts and Lobules

## I. Incidence and Predisposing Conditions

CYRIL TOKER, M.D.

### Abstract

Breast biopsies removed for benign disease from 2582 patients during the years 1946–1967 were reviewed. Dysplastic lesions of small-cell type were observed within 98 patients—an incidence of four percent. These lesions comprise a spectrum that extends from basal dysplasia to frank in situ carcinoma. They are most commonly encountered during the fifth decade, and their actual incidence probably exceeds the figure quoted. It is likely that many are overlooked through inadequate sampling of biopsy specimens, and through lack of familiarity with the morphologic alterations. Although dysplastic changes were observed within foci of sclerosing adenosis the evidence is insufficient for a correlation of these entities.

Although lobular carcinoma in situ of the breast is an established entity (1, 2), reports of long-term clinical behavior are few in number (3). The ensuing communications will document our considerable experience with this rare lesion at The Mount Sinai Hospital and will, furthermore, present additional perspectives hitherto unrecorded by previous writers on the subject.

*Material*

Breast biopsies removed from 2582 patients, and reported as showing no more than benign breast disease, were reviewed. This review covered the years 1946–1967 inclusive. Small-cell dysplasias found during this study were subdivided into 3 categories: Type I, dysplasia; Type II, indeterminate; and Type III, in situ carcinoma. Description and illustration of these categories will appear in succeeding accounts.

*Incidence* (Tables I, II)

The incidence of dysplastic lesions was four percent; changes were found within 98 of the 2582 patients biopsied. Bilateral lesions were encountered on one occasion. Distribution of the lesions among the three categories was as follows: Type I, 40 cases; Type II, 34 cases; Type III, 25 cases. These figures approximate the relative percentages. Subsequent to 1967 further examples have come to our attention, with a present total of 134 patients. The incidence of dysplastic lesions during this latter 5-year period cannot be computed, for the total number of patients biopsied is not known. However, more precise data are available for the period 9/71 to 11/72, during which time 20 out of 236 consecutive biopsies for benign disease showed dysplastic changes—an incidence of nine percent. This higher incidence probably reflects improved

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detection due to an increase in the number of sections taken from each specimen. Our current practice is to embed at least 5 tissue blocks from all breast biopsies in patients 40 years of age or more.

### *Incidence of Contralateral Invasive Carcinoma (Table III)*

Ten of the 134 patients had undergone prior contralateral mastectomy for invasive carcinoma, and three underwent simultaneous contralateral mastectomy for invasive tumor at the time of biopsy—a total incidence of ten percent. Data relating to the development of contralateral invasive carcinoma subsequent to the biopsy will be presented later in this series.

### *Age Incidence (Table IV)*

The highest incidence in all three categories was noted during the fifth decade. Examples were, however, encountered as early as the third and as late as the eighth decades.

### *Relationship to Preexisting Proliferative Disorders of the Breast*

We have observed 14 instances in which dysplasia or in situ carcinoma of lobules has occurred within foci showing the pattern of sclerosing adenosis. With regard to the possible relationship, if any, between sclerosing adenosis and mammary dysplasia, we find it impossible to make any statement on the basis of these findings. Such a statement would require a comparison between the

Table I

*Incidence of Small-Cell Lesions, 1946–1967*

| Number of patients | Number of patients with lesions | Incidence |
|---|---|---|
| 2582 | 98 | 4% |

Table II

*Relative Incidence of Type I, II, and III Lesions (1946–1967)*

| Type I | Type II | Type III | Total |
|---|---|---|---|
| 40 | 34 | 25 | 98* |

* 1 patient had bilateral lesions, and is included both in Type II and Type III categories.

Table III

*Incidence of Prior or Simultaneous Contralateral Mastectomy for Invasive Carcinoma (1946–1972)*

| Number of patients | Number of tumors | Incidence |
|---|---|---|
| 134 | 13 | 10% |

Table IV

*Age Incidence; 134 Cases of Small-Cell Dysplasia (1946–1972)*

| Age | Type I | Type II | Type III |
|---|---|---|---|
| 20–29 | | 1 | |
| 30–39 | 8 (15%) | 2 (4%) | 4 (11%) |
| 40–49 | 25 (46%) | 26 (56%) | 18 (49%) |
| 50–59 | 14 (26%) | 13 (29%) | 8 (22%) |
| 60–69 | 6 (11%) | 3 (6%) | 5 (14%) |
| 70–79 | | 1 | 1 |
| 80–89 | 1 | | 1 |
| Total* | 54 | 46 | 37 |

* 3 patients had bilateral lesions and are included within more than 1 category.

incidence of dysplasia in sclerosing adenosis and that of dysplasia independent of this change. Attempts to assess the incidence of sclerosing adenosis have been made during this study, but are dependent upon too many variables (such as, for example, the number of sections taken) to be reliable. Neither can we exclude the possibility that the fibrotic reaction characteristic of sclerosing adenosis may succeed, rather than precede, the dysplastic alteration. The problem must therefore remain sub judice, and we cannot now assert that patients with sclerosing adenosis are exposed to any greater hazard of mammary dysplasia than the remaining female population.

## Discussion

Small-cell dysplasia and in situ carcinoma are rare lesions. The incidence is, however, probably greater than the four percent observed during the period 1946—1967. During this period few sections were taken from each specimen, often no more than a single slide being prepared. Increasing the number of blocks per biopsy will undoubtedly increase the detection of dysplastic lesions, for these lesions may be found in only part of the total amount of tissue processed. It is, furthermore, likely that some lesions may have been overlooked, for the small-cell proliferations are of innocuous aspect, and may occur in isolated foci. With increasing experience and improved sampling it is to be anticipated that fewer lesions will pass undetected, and the nine percent incidence recorded over the last year may prove to be more accurate than the earlier figure.

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# Small-Cell Dysplasia and In Situ Carcinoma of Mammary Ducts and Lobules

## II. Normal Mucosal Structure and the Differentiation of Dysplastic from Nondysplastic Proliferations

CYRIL TOKER, M.D.

### Abstract

Distinction between dysplastic and nondysplastic proliferations of the mammary epithelium is qualitative, and is dependent upon the growth pattern. Dysplastic proliferations tend to uniformity of organization, with the production of solid cellular sheets or spherical glandular lumina. Nondysplastic masses, by contrast, are characterized by irregularity and disarray. They are essentially trabecular rather than diffuse, and their lumina are serpiginous rather than spherical. Differentiation of the serious from the innocuous small-cell lesions is dependent to a much greater extent upon these nuances of histologic arrangement than upon cytologic criteria.

The normal mammary tubule, whether it be acinus or duct, is lined by a double-layered epithelium (Fig. 1) (1, 2). The superficial layer is composed of columnar, or cuboidal, eosinophilic cells. The basal layer contains crescentic cells with clear cytoplasm. The eosinophilia, uniformity, and compactness of the superficial stratum contrast with the loose texture, variability, and light staining of the basal zone. The polarity of the cells forming these layers is, furthermore, dissimilar. Thus the axes of the superficial cells are perpendicular to the basement membrane, while the axes of many basal cells are parallel to that structure. Disturbance of normal mucosal stratification and polarity is characteristic of dysplastic epithelial proliferations, which impose a certain homogeneity upon the tubular lining.

Before the dysplastic changes are described, it is appropriate to define the designation 'small-cell.' Nuclear dimensions range from a diameter approximately equal to that of the erythrocyte—some 8 microns—to a size of some 15 microns. Smaller nuclei tend to pyknosis; larger nuclei exhibit a more lightly staining vesicular structure. Cytoplasm is usually scanty, but moderate amounts are present in some instances (Fig. 2). In many cells eccentric nuclei and a rather eosinophilic cytoplasm present an appearance resembling that of myo-epithelium (Fig. 2). Cell boundaries are often indistinct, only the nuclei being sharply defined (Fig. 3). Mitotic figures are sparse. Small-cell proliferations often contain cells of different size, but their configuration, usually spherical, is uniform, and the profound variations of nuclear shape and chromatin content observed within large-cell tumors, are lacking. Huge nuclei, abundant cytoplasm, pleiomorphism, and mitotic activity, all signalize the large-cell tumor. Admixture of large and small cells may occur. Cases in which large cells predominated have been excluded from this study.

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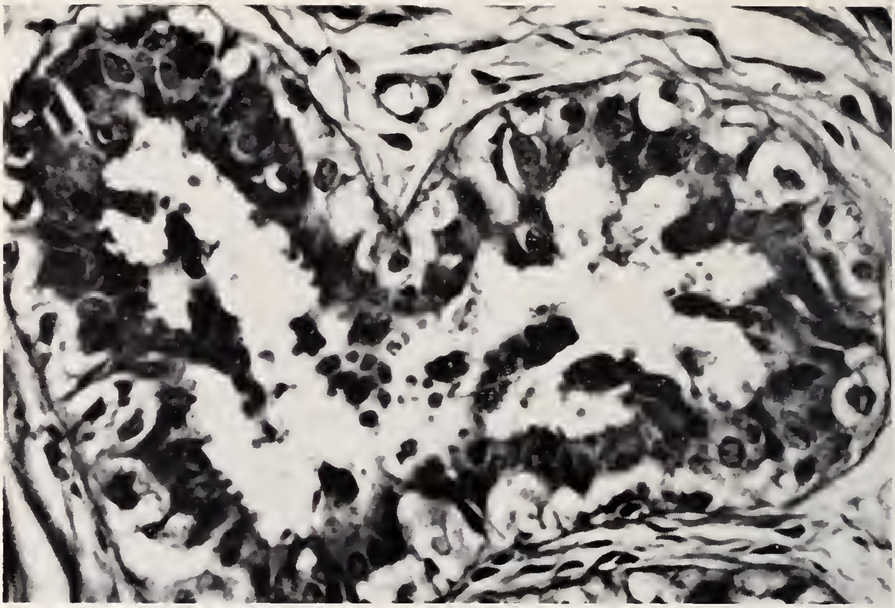

Fig. 1. Normal mammary acinus. The mucosa consists of 2 layers (H&E × 320).

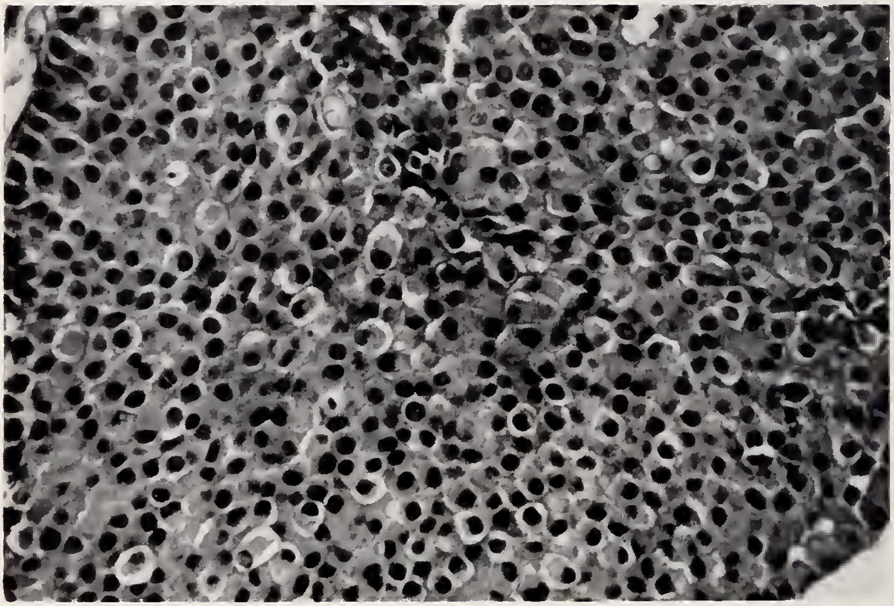

Fig. 2. Small-cell dysplasia involving a duct. A diffuse sheet of cells is seen. Eccentric nuclei and eosinophilic cytoplasm present, in some cells, an appearance similar to myoepithelium (H&E × 200).

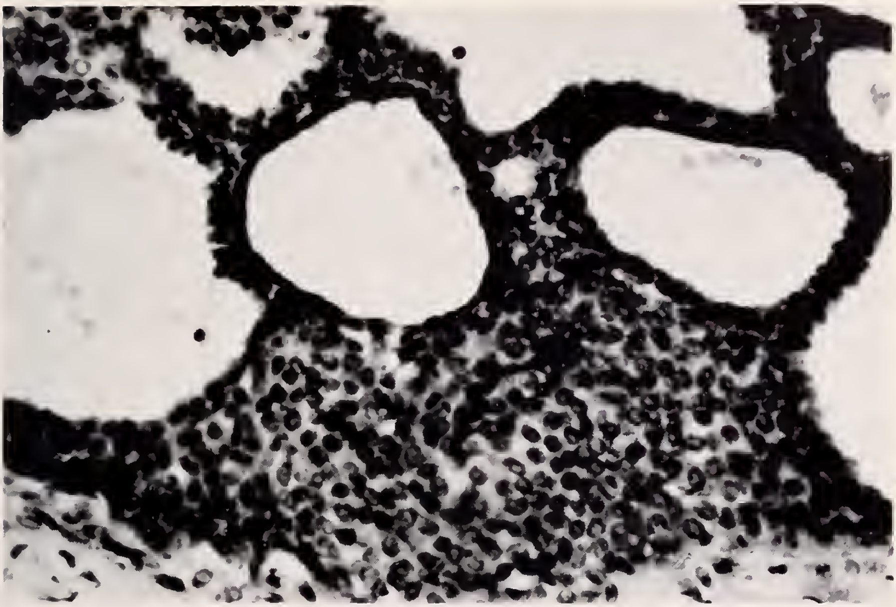

FIG. 3. Small-cell dysplasia involving a duct. The lesion is predominantly cribriform, but a solid sheet of cells is shown, in relation to the glandular lumina. Cell boundaries are indistinct (H&E × 200).

## *Distinction between Dysplastic and Nondysplastic Epithelial Proliferations*

Distinction between dysplastic and nondysplastic epithelial proliferations of the mammary tubules is qualitative, and is dependent upon the growth pattern. Within the dysplastic group, however, subdivision into categories reflecting the extent to which the disorder has progressed is predominantly, albeit not entirely, quantitative.

Both dysplastic and nondysplastic proliferations may be solid (aglandular), cribriform (glandular), or papillary. The papillary variety will not be considered in these discussions. The solid and glandular forms represent essentially the same process, and both are included within the small cell categories under consideration. They may occur in varying admixture (Fig. 3), but one pattern usually predominates. Such admixture may be seen within both dysplastic and nondysplastic masses. It is emphasized that the direction of the dysplastic multiplication, whether it be cribriform or solid, is toward uniformity, toward homogeneity, of the cellular mass. The nondysplastic theme, by contrast, is one of irregularity and disarray. Foci of calcification may be observed within both dysplastic and nondysplastic epithelial masses.

Within the solid type of dysplastic proliferation the cells form apolar sheets (Fig. 2). They may retain some elongation, but usually assume a polygonal or spherical shape. A diffuse mass, devoid of organization, is produced (Fig. 2). Nondysplastic proliferations of solid type, by contrast, exhibit anastomosing trabeculae separated by narrow clefts (Fig. 4). These trabeculae are placed

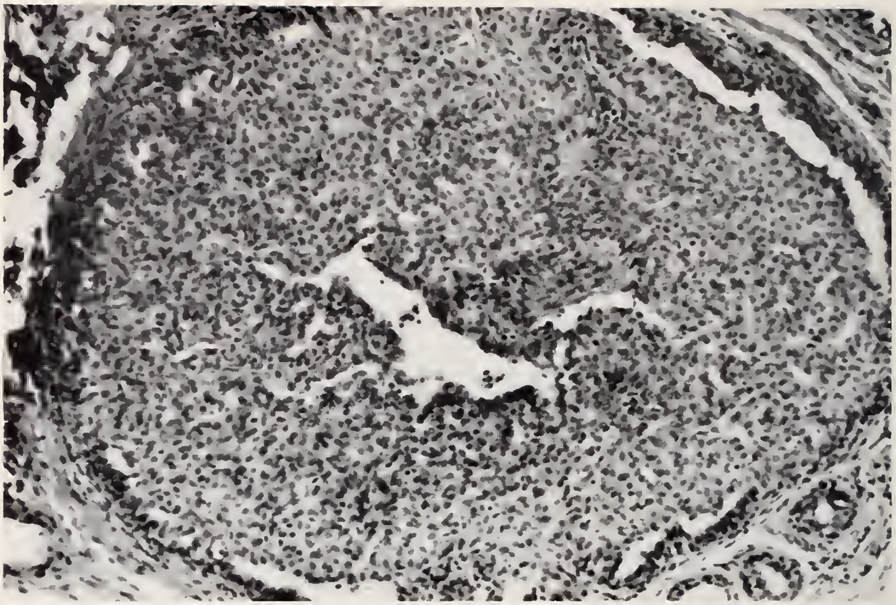

FIG. 4. Solid nondysplastic proliferation involving a duct. Anastomosing trabeculae are separated by narrow clefts (H&E × 80).

irregularly at various angles to one another, and are composed of columnar or polygonal cells arranged in single- or double-layered rows. Broad sheets are not seen.

This, then, is the essential distinction—the construction of the nondysplastic mass is fundamentally trabecular, while that of the dysplastic is fundamentally diffuse. Recognition of a trabecular construction is critical, for upon this recognition will depend the correct interpretation of a nondysplastic lesion. The trabecular composition of a crowded epithelial mass may, however, not be overt, and under these circumstances difficulty, and difference of opinion, may well result. Preparation of further sections may resolve the dilemma through the revelation of clefts, the presence of which will usually expose the trabecular nature of the lesion. It should be noted that dysplastic changes may supervene within a preexisting nondysplastic proliferation (Fig. 5).

Cribriform multiplications are more readily classified than their solid counterparts. The configuration of the glandular clefts is distinctive (Figs. 5, 6). Thus the dysplastic lumina tend to assume a spherical shape, while the nondysplastic form irregular, serpigenous, elongated channels. The intervening cells may, in either variety, be columnar (tall) and uniform, or small, polygonal, and variable in size. Within the nondysplastic lesion the intervening cells form single- or double-layered trabeculae. Sheets are not seen. The dysplastic mass, however, will invariably manifest, in addition to single- or double-layered cords, broader sheets interposed between the glandular lumina (Fig. 3).

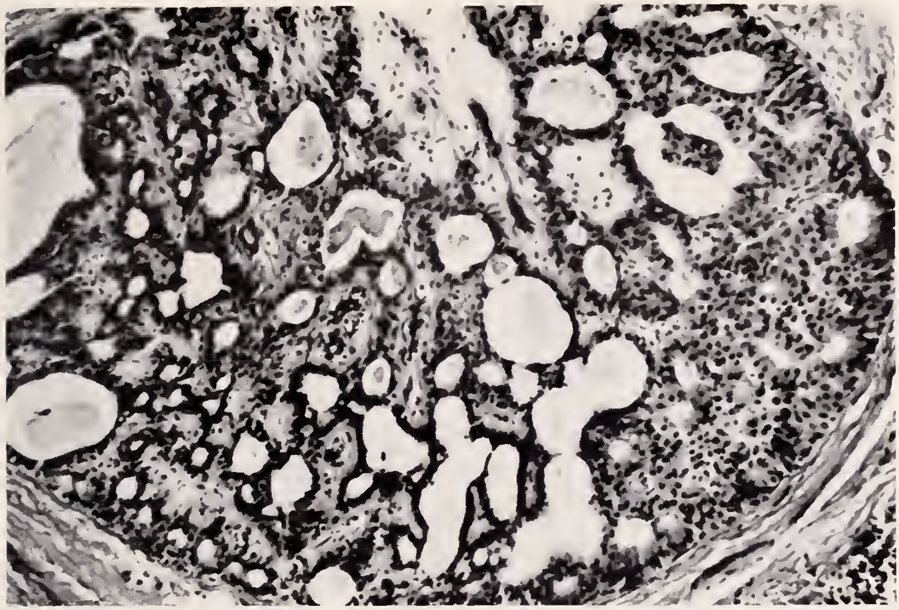

FIG. 5. Cribriform proliferation involving a duct. Dysplastic change has developed in a preexisting nondysplastic lesion. Uniform, spherical lumina seen on the right-hand side of the figure contrast with the irregular, serpigenous channels seen in the greater part of the micrograph (H&E × 80).

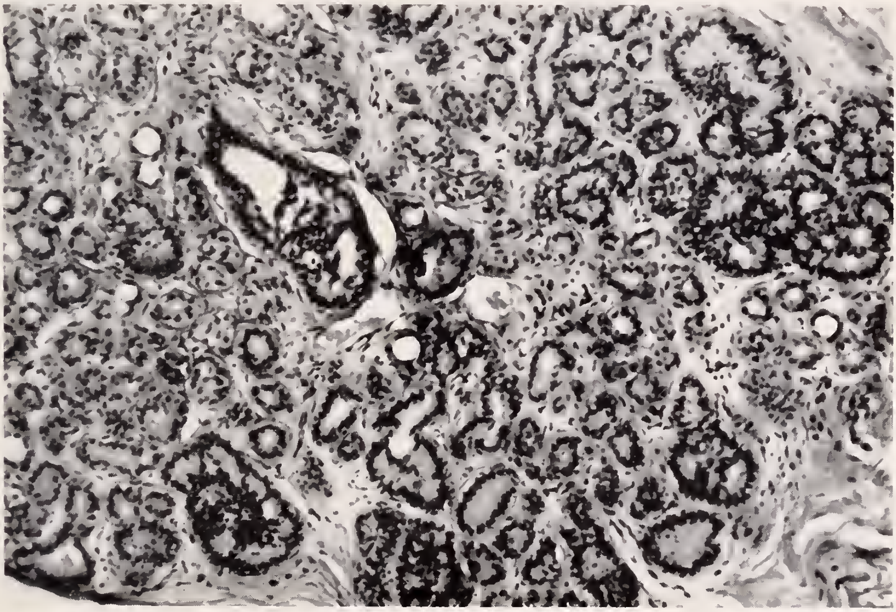

FIG. 6. Cribriform dysplasia affecting a lobule. Uniform, spherical lumina confer a honeycomb pattern upon the proliferating acini (H&E × 80).

It should be emphasized that these processes do not exhibit regional specificity. Thus the cribriform lesions may involve either lobules (Fig. 6) or ducts, or both in combination. Solid proliferations, similarly, may affect ducts (Fig. 2) or lobules, independently or in combination.

## Comment

The cribriform type of dysplastic epithelial proliferation presents an impressive picture that is distinctive and conspicuous. Differentiation from the nondysplastic glandular multiplications is usually not difficult, for the disparity between the two patterns is considerable and relates to parameters that are comparatively coarse—parameters that are, therefore, rather readily evaluated. The solid (aglandular) proliferations, by contrast, approximate each other more closely, and distinction between the dysplastic and nondysplastic processes is dependent upon more subtle shades of cellular organization. The problem here is more difficult, and this difficulty accounts, we believe, for the greater number of unrecognized solid dysplasias encountered in this review.

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# Small-Cell Dysplasia and In Situ Carcinoma of the Mammary Ducts and Lobules

## III. Categorization of Mammary Dysplasia

CYRIL TOKER, M.D.

### Abstract

Dysplastic epithelial proliferations of the mammary ducts and lobules present a continuous gradation that extends from the earliest basal alteration to frank in situ carcinoma. This gradation has been subdivided into 3 categories that have been designated as Type I (dysplasia), Type II (indeterminate), and Type III (in situ carcinoma). This categorization is applicable equally to both the solid and the cribriform varieties, and is dependent upon quantitative factors relating to the extent of the epithelial multiplication.

The dysplastic changes with which we are concerned represent a continuous and insensible gradation that extends from the earliest basal alteration to frank is situ carcinoma. This continuum has been divided, on a basis that is quantitative rather than qualitative, into 3 categories, which have been designated as Types I, II, and III, respectively. Even within the categories that we have defined, however, a continuous gradation may be observed, and it should be emphasized that the individual members of each category are not necessarily identical. It should, furthermore, be observed that varying degrees of dysplasia may be found within the same biopsy or mastectomy specimen. In this regard it may be noted that increasing the number of sections prepared may improve detection of more advanced dysplastic changes.

These lesions, whether solid or cribriform, may arise within either lobules or ducts, independently or in combination. The solid and cribriform variants represent, essentially, facets of a single process, and both have been included within the small-cell categories under consideration. In this series the cribriform variety was in the minority, there being but eight instances in the Type I, one in the Type II, and five in the Type III categories, respectively (1).

### *Solid Dysplasia*

### *Type I Dysplasia*

It is probable that the dysplastic change invariably commences within the basal layer (Fig. 1), although artefacts of sectioning may obscure the site of initial proliferation. At low magnification the affected channel displays abnormal cellularity, often distinctly basal in location. It should be emphasized that detection of the dysplastic tubule is most readily accomplished under very low power objectives (e.g., x 2.5) at which magnification the crowded channel contrasts noticeably with the surrounding normal mammary ducts and lobules. An intact superficial layer of the mucosa may persist as a covering for the

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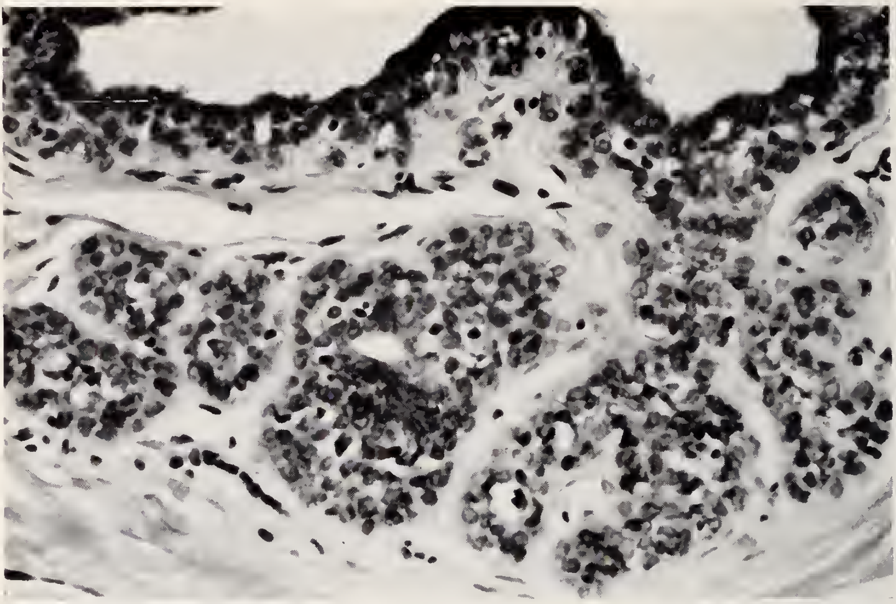

Fig. 1. Type I dysplasia, solid. Sheets of cells occupy the basal mucosal zone (H&E × 200).

proliferating basal cells which may, on occasion, form no more than isolated clusters within the basal layer of an otherwise intact and undistorted mucosa. Alternatively, the superficial stratum may have disappeared, and both the superficial and the basal layers may come to resemble one another, being composed of uniform polygonal or spherical cells (Fig. 2). Normal mucosal contrasts are effaced. The proliferating cells may retain some elongation, but they are beginning to form sheets devoid of any trabeculation. With further progression the cells assume a spherical or polygonal shape, and distension of the channel by the cellular mass may commence (Figure 1).

The condition of the lumen varies. This is usually patent. In this regard the misleading effects of tangential sectioning must be avoided. A section passing tangentially through a purely basal mass may convey a deceptive impression of advanced cellular proliferation. Attention should, therefore, always be directed toward channels within which the lumen is visualised, for under these circumstances the correct anatomical relationships may be determined, and the limited, basal nature of the proliferation may be appreciated. Where doubt persists further sections will often resolve the issue. The lumen may not invariably be discernible, however, for some channels may be collapsed (Fig. 2). The limited nature of the process may, nevertheless, still be recognized, for the duct or lobule will retain a narrow elongated shape, frequently manifesting a characteristic angular, tortuous course (Fig. 2). This angulation, this tortuosity, is progressively dissipated in the more advanced proliferations as cellular accumulation distends the channel. Dissociation of cells (i.e., separation and isolation from one another) is slight or absent in Type I lesions.

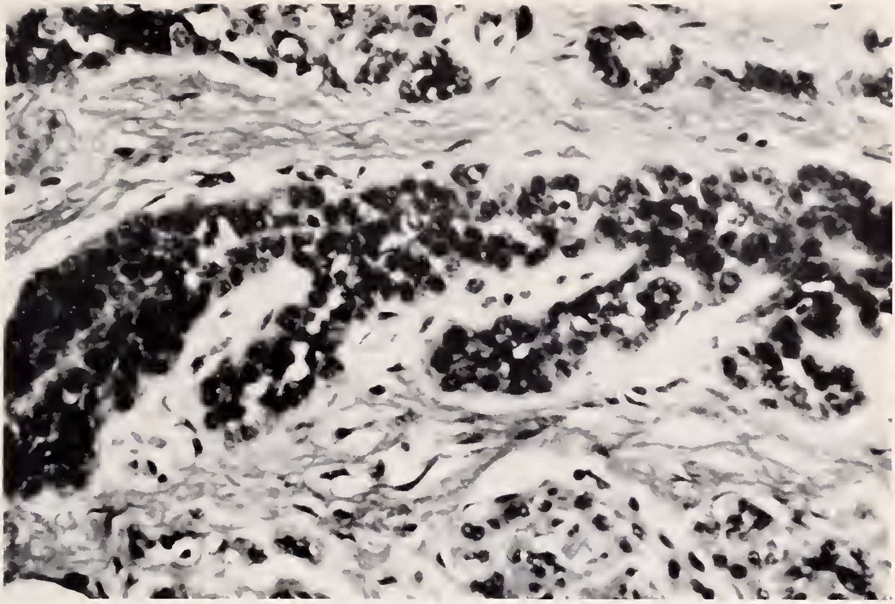

FIG. 2. Type I dysplasia, solid. The normal mucosal layers are replaced by a homogeneous mass of cells. The channel is collapsed. Distension is not seen (H&E × 200).

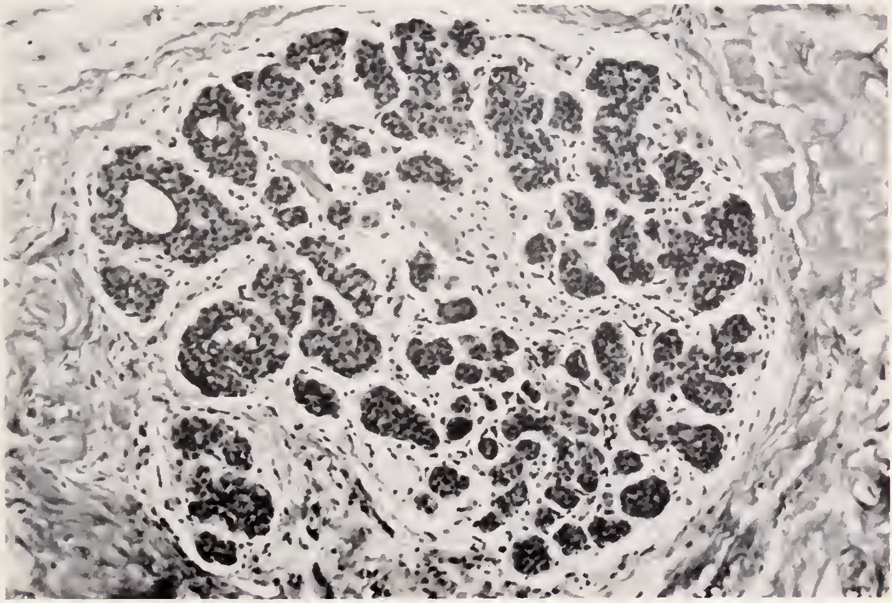

FIG. 3. Type II dysplasia, solid. The acini are crowded by masses of cells. Angularity of outline is still evident (H&E × 80).

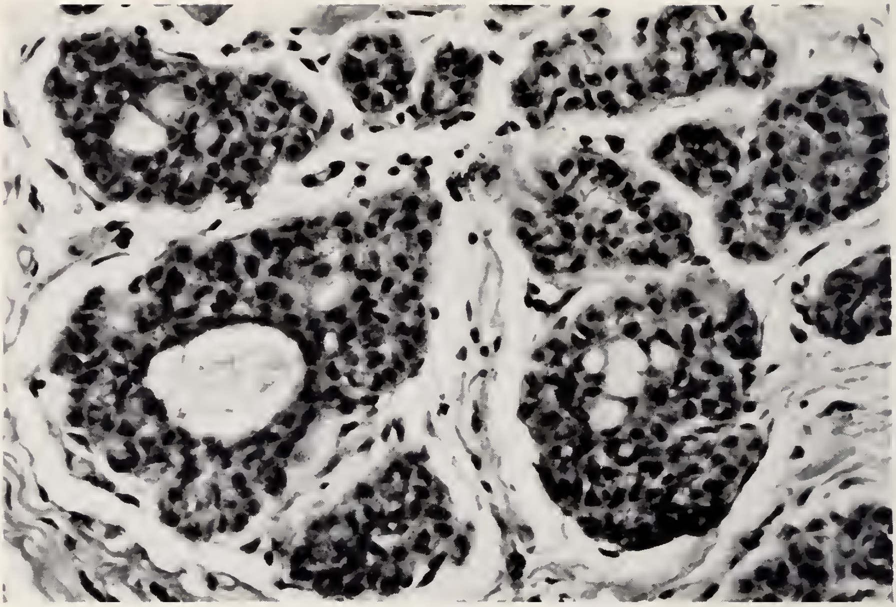

Fig. 4. Type II dysplasia, solid. Irregularly distended acini are filled by sheets of cells. Some lumina persist (H&E × 200).

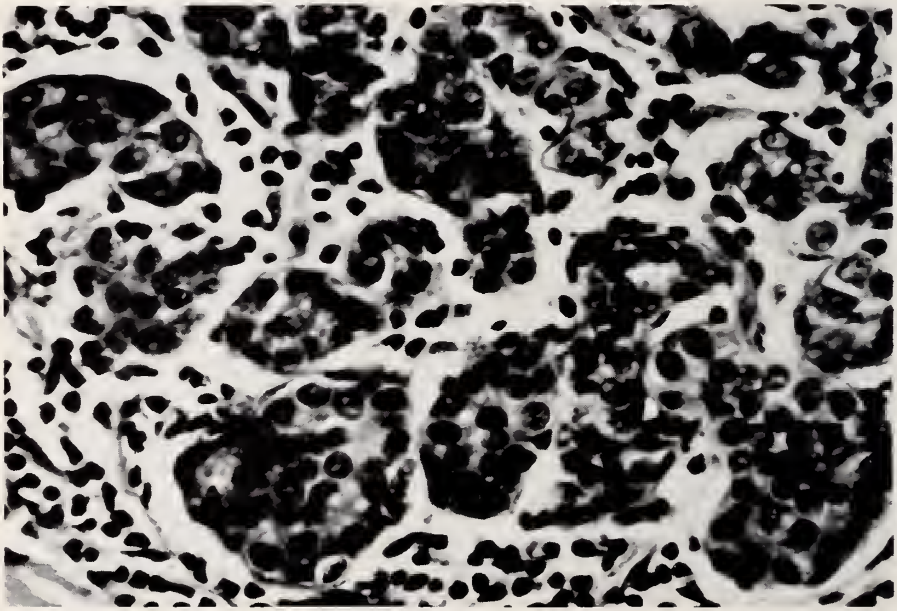

Fig. 5. Type II dysplasia, solid. The cells form small rosettes, indicative of attempts to produce lumina (H&E × 320).

### *Type II Dysplasia*

Type II lesions demonstrate a quantitative progression of the dysplastic proliferation. At low magnification (Fig. 3) the crowded ducts or lobules stand out darkly. Distension of the tubules is apparent, but some angularity and tortuosity of outline persist (Figs. 3, 4). The polygonal or spherical cells are disposed in sheets. Within these apolar sheets the nuclei may be aligned in circular rows or small rosettes (Fig. 5)—characteristic formations indicative of attempts to form lumina. These formations should not be confused with benign trabeculation. The lumen of the channel is often preserved, but may have been occluded. Normal mucosal architecture has been destroyed, although an attenuated superficial layer may survive atop the mass of cells. Dissociation of cells is limited.

### *Type III Dysplasia*

Type III dysplasia represents the ultimate florid expression of the dysplastic process. Tubules, distended by apolar sheets of cells, present a globular outline (Figures 6, 7). Their lumina are often occluded, but may persist in some instances. Remnants of the normal superficial mucosal layer may yet surmount the cellular mass, but usually no vestige of the normal lining is discernible. Dissociation of cells is often, but not invariably, pronounced—the spherical or polygonal cells floating, widely separated from one another, within the loosely textured sheets (Figure 8).

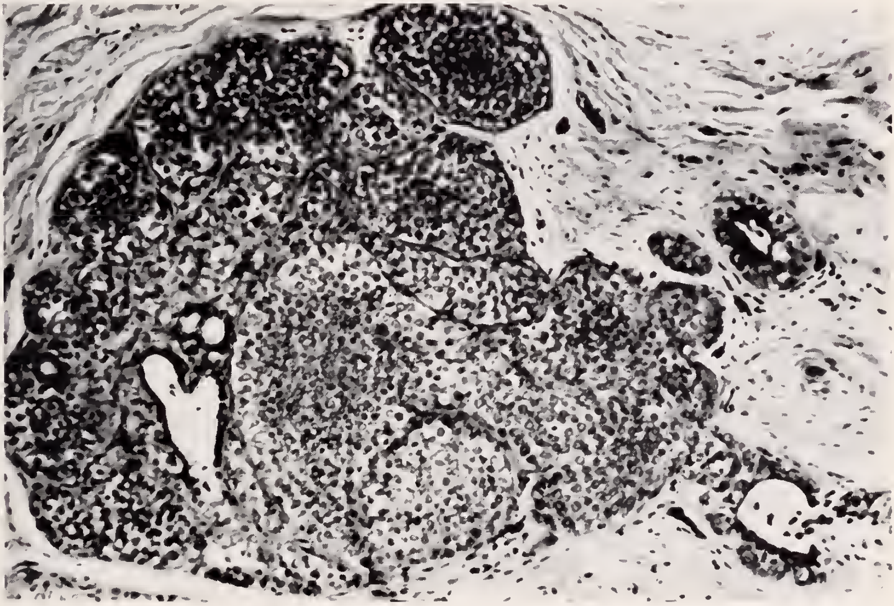

FIG. 6. Type III dysplasia, solid. Distended acini are filled by masses of cells (H&E × 80).

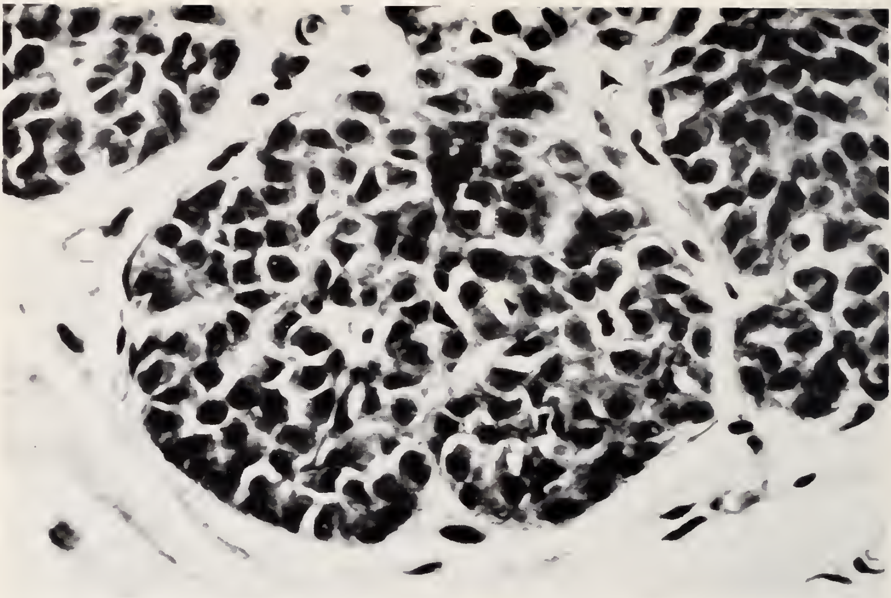

FIG. 7. Type III dysplasia, solid. A distended globular acinus is filled by a sheet of small cells. (H&E × 320).

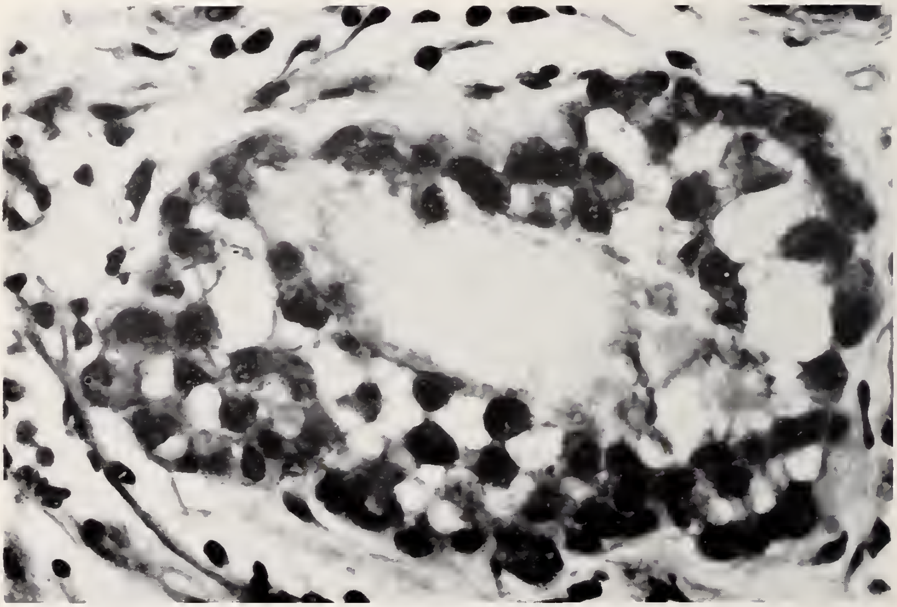

FIG. 8. Type III dysplasia, solid. Dissociation of cells is pronounced (H&E × 320).

### *Cribriform Dysplasia*

Essentially similar principles apply to the categorization of the cribriform dysplasias. Type I lesions (Fig. 9) display cribriform lumina within the cellular sheets. The number of lumina is small, and the channels are not distended. Type II lesions (Fig. 10) manifest progression of the process with some degree of distension and an increase in the number of lumina. However, some angularity of the tubular outline may yet persist. The Type III lesion (Fig. 11) exhibits large numbers of essentially spherical lumina. The distended channel assumes a circular outline. Central necrosis may be apparent. The cribriform pattern may, in all three groups, dominate the morphologic picture, or may coexist in varying measure with the solid (aglandular) form.

### *Cylindromatous Dysplasia*

This extremely rare dysplastic variant (Fig. 12) has been observed on but three occasions. Within cylindromatous proliferations, in contrast to the true glandular form, lumina are produced by the disintegration of sequestrated connective tissue cores that have been secluded within the epithelial mass. This process, while resulting in a cribriform pattern, differs from primary tubule formation as seen in the more common glandular (cribriform) dysplasia. It is to be presumed that the cylindromatous dysplasia may progress, in some instances, to an invasive cylindroma, but information on this aspect is

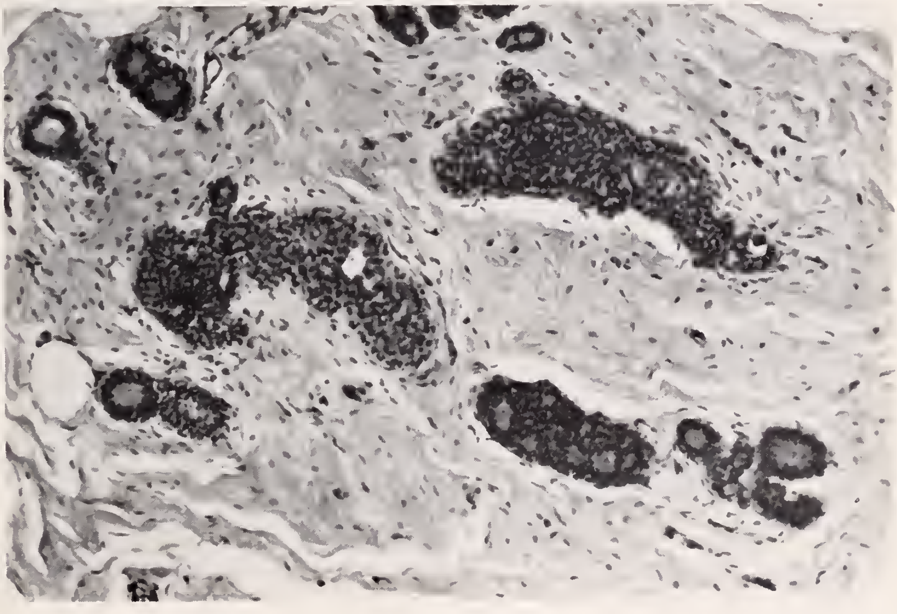

FIG. 9. Type I dysplasia, cribriform. Spherical lumina are seen within the cellular masses. The ducts are not distended (H&E × 80).

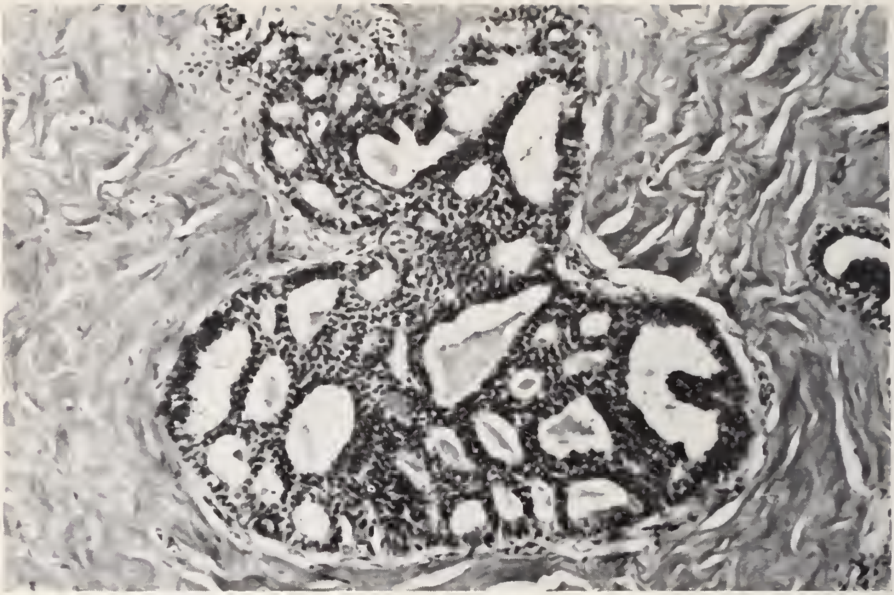

Fig. 10. Type II dysplasia, cribriform. The duct shows some distension (H&E × 80).

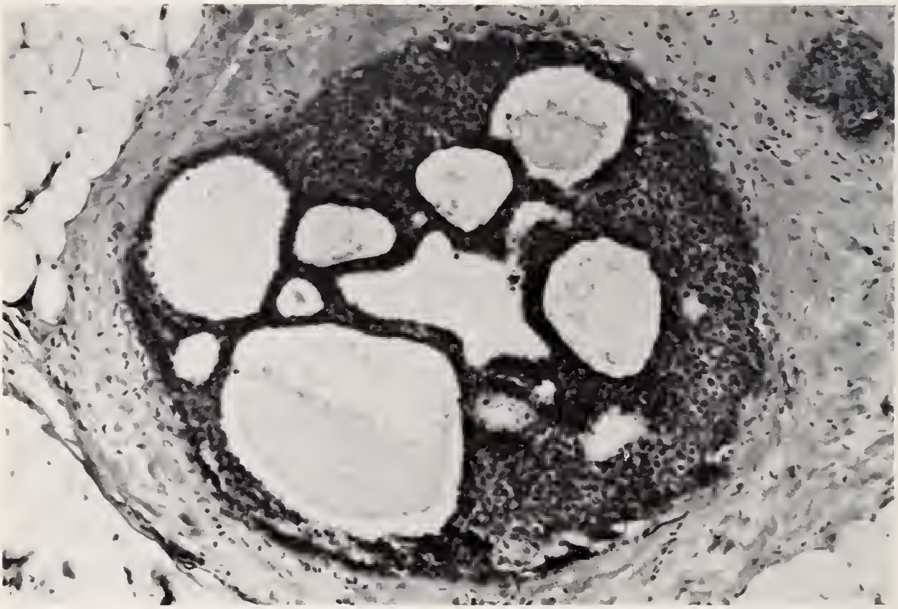

Fig. 11. Type III dysplasia, cribriform. The duct is distended: the outline is circular (H&E × 80).

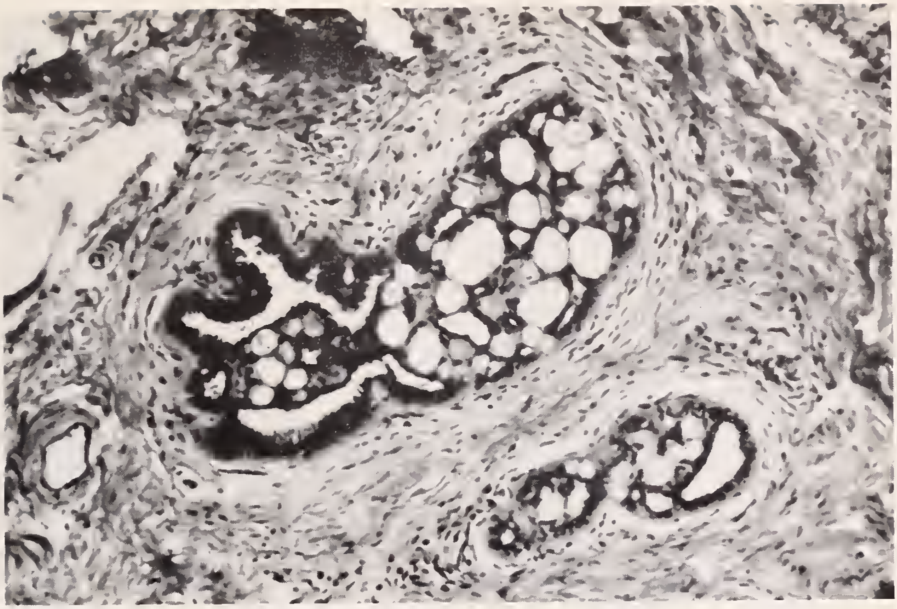

FIG. 12. Cylindromatous dysplasia. Stromal (collagenous) cores sequestrated within the epithelial masses degenerate, with the secondary production of spherical lumina (H&E × 80).

nonexistent. One of our patients in this category has been followed for 8 years, without further sequelae. The other two examples are too recent for comment.

## Discussion

Evaluation of in situ lesions is difficult and tendentious. Lacking the finite evidence of invasion, interpretation is often diverse. Criteria are not readily defined. It is, nevertheless, imperative that dysplastic mammary proliferations be graded, as are dysplastic proliferations elsewhere in the body. The prognostic and therapeutic implications are evident.

Such a categorization has been attempted herein. It is certainly true that others may disagree with our classification of individual lesions, and further clarification and definition of criteria is to be anticipated. We are, however, convinced of the general validity of the approach that has been adopted, and would note the correlation that has been found between the grading of the lesions—undertaken without any attention to subsequent clinical developments—and the incidence of later invasive carcinoma. The details of this correlation will be presented in the succeeding section of this series.

Types I and III do, in the main, provide little difficulty. In conventional terms we may designate Type I as atypical hyperplasia or dysplasia, and Type III as in situ carcinoma. The true nature of the Type II proliferation is uncertain. Do these intermediate lesions represent early carcinomas, or do they reflect a severe dysplasia not yet capable of undertaking invasive growth? We are unable to resolve this dilemma, and have therefore adopted the noncommittal title of Type II, thereby avoiding the clinical impact of a malignant diagnosis.

The titles that are applied to lesions are essentially inconsequential. They have significance solely in terms of clinical behavior. Our objective has been to convey to the surgeon some estimate of the hazard to which the patient in each category is exposed irrespective of the designation that may be applied to the category in question. If this hazard does indeed bear any relationship to the extent of the morphological alteration, it is apparent that a system of classification is mandatory. We believe that the classification presented can provide a basis for further evaluation of dysplastic lesions.

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# Small-Cell Dysplasia and In Situ Carcinoma of the Mammary Ducts and Lobules

## IV. Development of Invasive Carcinoma

CYRIL TOKER, M.D.

### Abstract

Dysplastic small-cell proliferations of the mammary ducts and lobules have been subdivided into 3 categories, designated as Types I (dysplasia), II (indeterminate), and III (in situ carcinoma), respectively, according to the degree of the atypical change. Within the Type I group a 4% incidence of subsequent, ipsilateral, invasive carcinoma was noted at 10 years, and a 12% incidence at 15 years. Within the Type II group the incidence of ipsilateral invasive carcinoma was 14% at 10 years and 25% at 15 years. Within the Type III group the incidence of ipsilateral invasive carcinoma was 21% at 10 years and 45% at 15 years. Differentiation of frank in situ carcinoma from lesser degrees of atypia has both prognostic and therapeutic significance.

In order to determine the incidence of subsequent invasive carcinoma in both the ipsilateral and contralateral breasts of patients with dysplastic lesions, follow-up information was sought in all cases; irrespective of whether the initial procedure had been local excision or mastectomy. Table I presents the results of this endeavor with regard to patients who were treated solely by local excision during the years 1946–1967. It will be noted that the great majority of patients were located. The follow-up is continuing on a yearly basis for each patient. Thus far six cases in this group have been lost (Table II)—five through death from other causes, and one through discontinuation of contact by the patient. All patients who were subjected to immediate mastectomy have been located and are being followed for evaluation of contralateral carcinomas. It should be noted that subcutaneous mastectomy may be incomplete. One of our patients, subjected to subcutaneous mastectomy, developed an infiltrating carcinoma on that side after 13 years. However, subcutaneous mastectomy is presumed to have been complete if ipsilateral carcinoma has not developed, and these patients have been excluded from computations relating to the incidence of subsequent ipsilateral cancers.

### *Incidence of Subsequent Ipsilateral Invasive Carcinoma*

Follow-up data relating to lesions removed by local excision during the period 1946–1967 are presented in Table III. Patients who have developed ipsilateral invasive carcinoma have reached the end stage of the local disease process, and are included in each summation subsequent to the year in which the carcinoma developed. It may be noted that at 10 years the incidence of ipsilateral invasive carcinoma was 4% in Type I, 14% in Type II, and 21% in Type III dysplasias respectively. At 15 years the comparable figures were 12%

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Table I

*Follow-Up Rate among Patients Subjected Only To Local Excision of Dysplastic Lesion 1946–1967*

| | Type I | Type II | Type III |
|---|---|---|---|
| Traced | 35 | 30 | 19 |
| Not traced | 5 | 3 | 0 |
| Total | 40 | 33 | 19 |
| %FU | 88 | 91 | 100 |

Table II

*Cases Lost in the 5–15 Year Follow-Up, from Death or Disappearance (1946–1967 group)*
*(Figures in parentheses indicate years of available follow-up)*

| Type I | Type II | Type III | Total |
|---|---|---|---|
| Death 2<br>(8, 10) | Death 3<br>(5, 5, 7) | Death 0 | Death 5 |
| Loss 1<br>(8) | Loss 0 | Loss 0 | Loss 1 |

Table III

*Development of Ipsilateral Carcinoma (Lesions Removed 1946–1967)*

| Follow-up years | Type I | | | Type II | | | Type III | | | Total cases (all types) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. cases | No. ca. | % ca. | No. cases | No. ca. | % ca. | No. cases | No. ca. | % ca. | No. cases | No. ca. | % ca. |
| 5 | 35 | 0 | 0 | 30 | 2 | 7% | 19 | 1 | 5% | 84 | 3 | 4% |
| 10 | 23 | 1 | 4% | 22 | 3 | 14% | 14 | 3 | 21% | 59 | 7 | 12% |
| 15 | 17 | 2 | 12% | 16 | 4 | 25% | 11 | 5 | 45% | 44 | 11 | 25% |

25% and 45% respectively. The total incidence of subsequent ipsilateral invasive carcinoma in the entire group, including all 3 categories, was 25% at 15 years—a figure close to that previously reported (1).

## *Incidence of Contralateral Invasive Carcinoma*

As has been previously indicated (2) a 10% incidence of antecedent or coincident contralateral invasive carcinoma has been observed in patients with dysplastic small-cell lesions. With regard to the development of subsequent contralateral invasive carcinoma in the follow up group, 1946–1967, (Table IV) a direct comparison is rendered difficult by the fact that five patients had undergone contralateral mastectomy prior to the detection of the dysplastic lesion in the opposite breast. It will be noted that only three patients have developed subsequent contralateral invasive carcinomas in this group. However the total number of contralateral tumors in this group, both preceding and succeeding detection of the dysplastic lesion on the opposite side, was eight—a number not appreciably less than the total of 11 cancers that have developed thus far in the ipsilateral (dysplastic) breasts. It would, therefore, appear that the incidence of contralateral invasive carcinoma is not significantly below that of ipsilateral invasive cancer. However a somewhat greater number of these contralateral growths precede than succeed detection of the noninvasive dysplasia in the opposite breast. It may, finally, be noted (Table V) that four patients in the follow-up group have developed bilateral invasive carcinomas.

### *Time of Development of Carcinoma*

Table VI presents the times of removal of ipsilateral invasive carcinomas. The period interposed between detection of the dysplastic lesion and the detection of invasive carcinoma was quite variable, ranging from 5 months to 13 years. The invasive tumors were removed at ages that varied between 51 and 71 years.

### *Relationship between the Cytology of the Dysplastic Lesion and Subsequent Invasive Carcinoma*

Nuclear sizes within the dysplastic group ranged from small (approximately 8 microns) (Fig. 1) to medium (some 15 microns) (Fig. 2). The smaller nuclei were often hyperchromatic, whereas the medium-sized nuclei more commonly exhibited a lighter, vesicular structure. As indicated in Table VII, both small and medium sized lesions exhibited an equivalent propensity for evolution into subsequent invasive carcinoma.

Table IV

*Contralateral Invasive Carcinoma: Relationship to Detection of Dysplasia in Opposite Breast (Years in Parentheses)*

| | Type I | Type II | Type III |
|---|---|---|---|
| Preceding | 3 (2, 2, 12) | 2 (2, 16) | |
| Succeeding | 1 (14) | 1 (11) | 1 (7) |
| Total | 4 | 3 | 1 |

Table V

*Bilateral Invasive Carcinoma: Relationship to Detection of the Mammary Dysplasia (Years in Parentheses)*

| | Type I | Type II | Type III |
|---|---|---|---|
| Succeeding (both breasts) | | 1 (11) | 1 (7) |
| Preceding (Contralat.) | 1 (2)* | 1 (16)† | |

* Ipsilateral breast removed 13 years later.

† Ipsilateral breast removed 16½ years later.

Table VI

*Subsequent Ipsilateral Invasive Carcinoma*
*Years after Detection of Dysplasia*
*(Age at Detection of Dysplasia in Parentheses)*

| Type I | Type II | Type III |
|---|---|---|
| 8 (50) | 5 months (61) | 2 (53) |
| 11 (49) | 3 (50) | 7 (47) |
| | 7 (45) | 7 (64) |
| | 11 (40) | 11 (50) |
| | | 13 (45) |

Table VII

*Nuclear Size of Dysplastic Lesions That Subsequently Developed Invasive Carcinomas (Ipsilateral)*

| | Type I | Type II | Type III | Total |
|---|---|---|---|---|
| Small | 2 | 1 | 1 | 4 |
| Mixed (Small & medium) | | 2 | 2 | 4 |
| Medium | | 1 | 2 | 3 |

## *Cytology and Histology of the Subsequent Invasive Carcinoma*

Five of the subsequent carcinomas were small-celled (Fig. 3), 3 were mixed (containing both small and large cells), and 2 were large-celled (Table VIII). Within one patient, who has not yet developed an invasive growth, extensive Type III small-cell proliferation was accompanied by a large-cell dysplasia in both ducts and lobules. Within a second patient, who presented with a Type I small-cell lesion, re-biopsy of the same breast after 7 years revealed a large-cell intraductal dysplasia (Fig. 4). Evidently the carcinogenic stimulus may produce the small cells alone, or both the small and large cells in combination. Subsequently developing invasive carcinomas were variously trabecular or tubular in structure. Neither pattern predominated. It is, therefore, impossible, in any given small-cell lesion, to predict either the cytology or the histology of a subsequently developing invasive carcinoma.

## *Relationship between Extent of the Dysplastic Change and Development of Carcinoma*

No relationship has been found between the number of lobules or ducts affected and the subsequent incidence of carcinoma. Carcinomas have developed in patients who exhibited highly restricted involvement in the original biopsy. Conversely, patients with extensive lesions have thus far been free of sequelae.

## Comment

The majority of patients with dysplastic small cell proliferations have not thus far developed invasive carcinomas in either the affected or the contralat-

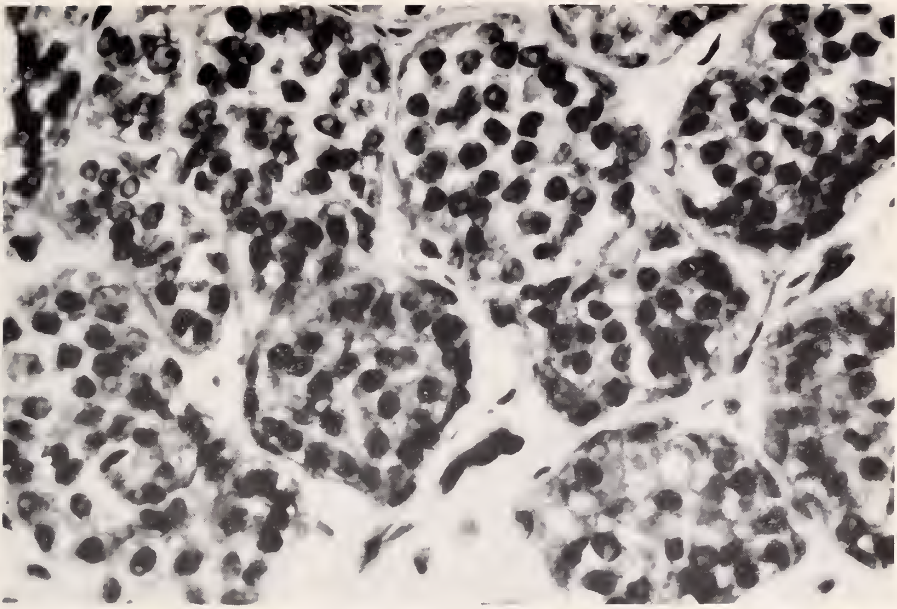

FIG. 1. Dysplastic lesion containing cells with small, hyperchromatic nuclei (H&E × 320).

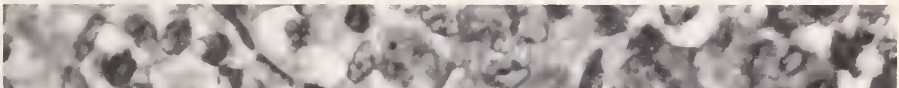

FIG. 2. Dysplastic lesion composed of cells with medium-sized, vesicular nuclei (H&E × 320).

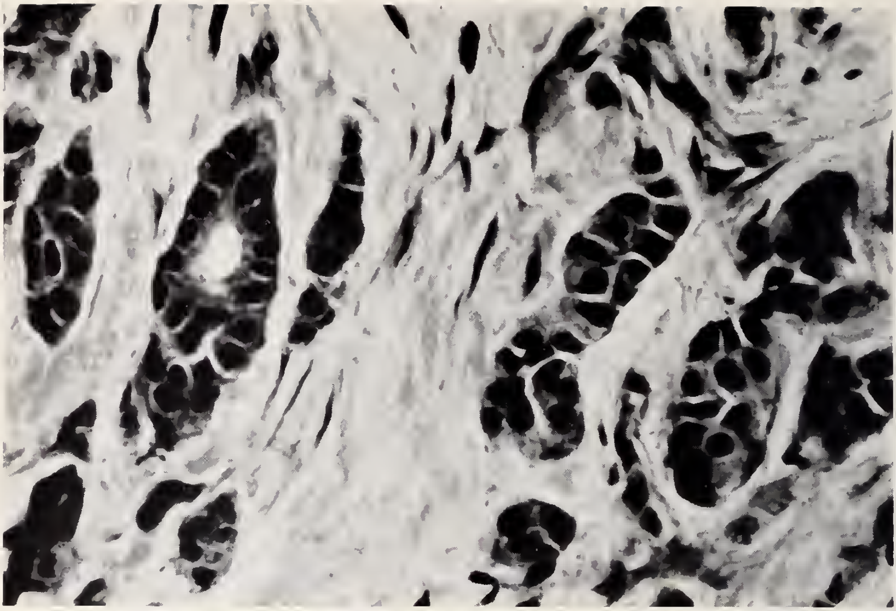

FIG. 3. Invasive small-cell carcinoma showing both tubular and trabecular structures (H&E × 320).

Table VIII

*Cytology of Subsequent Invasive Carcinoma*

| | Type I | Type II | Type III | Total* |
|---|---|---|---|---|
| Small cell | 1 | 1 | 3 | 5 |
| Mixed (Small & large cell) | 1 | 1 | 1 | 3 |
| Large cell | | 2 | | 2 |

* Sections on 1 case (Type III) not available.

eral breast. With regard to the affected breast it is apparent that there are two possible explanations for this finding, viz., that the diseased tissue had been entirely removed by the local excision, or secondly, that any residual dysplastic material regressed or remained inert. The development of invasive cancers in cases with lesser degrees of dysplasia presumably reflects either progression of the lesser to a more advanced degree, and finally, to infiltration; or, alternatively, to incomplete representation of the lesion in the original biopsy.

These possibilities notwithstanding, the surgeon is confronted by the necessity for decision when biopsy reveals a dysplastic mammary lesion. Our objective has been to convey to the surgeon some estimate of the hazard to which the patient in each category is exposed. It would appear that progression of the morphologic alterations is accompanied by an increase in the incidence of subsequent ipsilateral invasive carcinoma. It is not our purpose to make

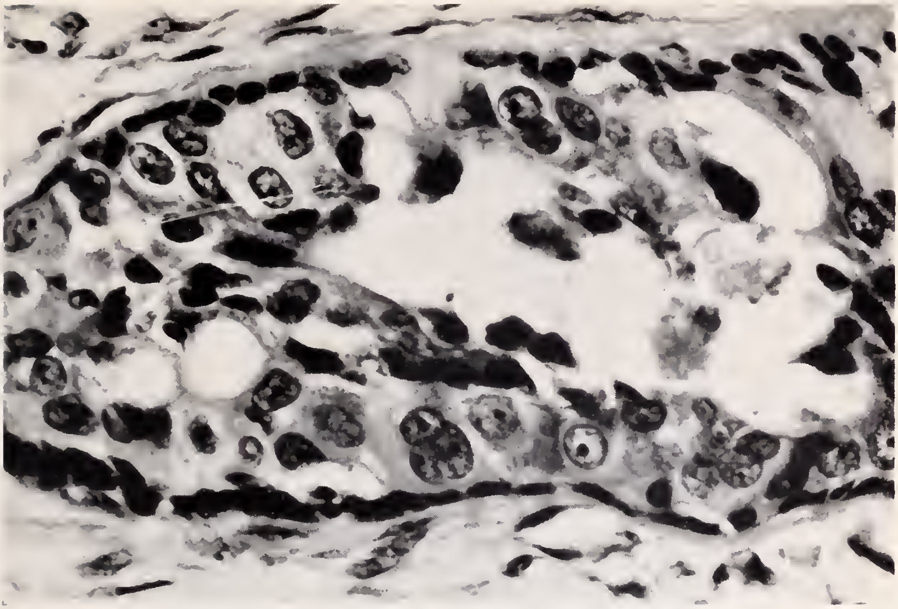

Fig. 4. Large-cell dysplasia developing in a patient with a known (preceding) small-cell lesion. Cell and nuclear sizes may be compared to those illustrated in the first 3 figures (H&E × 320).

recommendations with regard to the therapy of the respective categories. But it is our belief that the patient, as well as the surgeon, should share in the therapeutic decision. A 50% hazard of invasion after 15 years may be acceptable to one patient, a 10% hazard at 20 years may be unacceptable to another. We do not agree that this judgment resides solely within the domain of the surgeon. His major obligation is to inform the patient, and we have endeavored, via this study, to provide him with the wherewithal to do so.

## Acknowledgment

The author is indebted to the many surgeons and internists on the Mount Sinai Hospital Staff, who have so generously provided follow-up information on their cases. He owes a particular debt of gratitude to Dr. Eugene Friedman and Dr. Gerson Lesnick, whose patients formed a considerable proportion of the cases studied, for their help.

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# NEUROLOGIC NOTES

## Disturbance of the Visual Fields in Cerebral Vascular Malformations

*Submitted by*

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The clinical presentations of vascular malformations are varied. The common symptoms are seizures, hemimotor and hemisensory syndromes, and less frequently homonymous visual field defects (1–5). As a rule the latter are due to occipital lobe defects. Disturbances of the visual fields due to anterior lesions of the visual pathways are rare. Herein we present four such cases.

### Case Reports

*Case No. 1:* PL was a 28-year-old woman admitted to The Mount Sinai Hospital with a two-month history of 60-second episodes of technicolor spots starting in the middle of the left visual field and traveling slowly in a horizontal line into the right field and passing slowly out of the field on the right.

She has had intermittent bifrontal headaches over the previous year. At age of thirteen she had a severe excruciating diffuse headache which was associated with photophobia and a stiff neck. This lasted for a number of hours.

The neurologic examination was entirely normal except for a right lower quadrant homonymous visual field defect. Skull roentgenograms were normal. EEG and brain scan were not done. Left carotid arteriogram revealed a large vascular malformation in the left parietal area.

*Case No. 2:* LZ was a 54-year-old woman admitted to The Mount Sinai Hospital with the history of gradual neurological deterioration over many years. Apparently she had been well as a child and able to complete the eighth grade. After this she became less communicative and had deterioration in her intellectual functioning. She became increasingly more dependent for care on her mother and in the last twenty years had become virtually hidden away by her mother. In the last ten or fifteen years she had developed a right hemiparesis and she had grand mal seizures after reaching the age of thirty. At times these started in the right upper extremity. These had increased in intensity and frequency in the previous five years.

On examination she was awake but lethargic. She did not have spontaneous speech, and when she responded to direct questioning it became apparent that she had a markedly limited vocabulary. She had difficulty naming objects. She had a right homonymous field defect. She had difficulty maintaining gaze to the right. There was right facial weakness. There was flaccid paresis of the right arm and leg. There was a loud bruit heard over the left forehead, but not in the neck. Roentgenograms of the skull showed calcification in the left frontal area. Brain scan showed area of increased activity in this same area. Left carotid and right brachial arteriogram showed a large arteriovenous malformation running the whole length of the left cerebral hemisphere and filling from both the right and left side.

In the hospital the patient was started on anticonvulsant medication, and there was sig-

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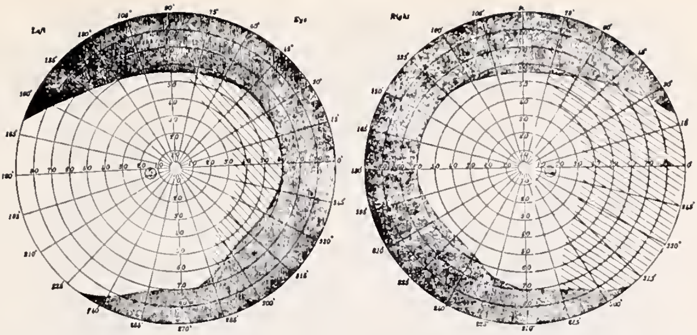

FIG. 1. Visual fields in the second patient showing an incomplete right homonymous field defect.

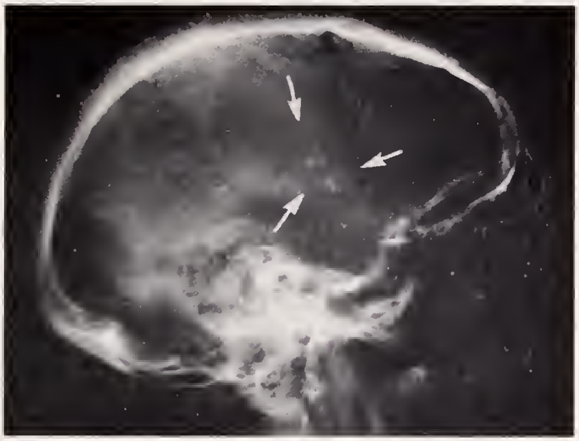

FIG. 2. Skull x-rays in the third patient with calcification in the center of the malformation.

nificant improvement in the level of consciousness, and some improvement in her intellectual functioning.

*Case No. 3:* CR was a 28-year-old man who was admitted with a history of difficulty in reading "because the letters on the left side were not clear." This had developed gradually over the last six months. He also had developed gradual difficulty in getting about because he was getting lost. He had several grand mal seizures four years previously, but these had not recurred since.

On examination the patient showed a mild organic mental syndrome, in that he made mistakes both in spelling and calculations. He had a homonymous left field defect. The remainder of the neurologic examination was normal. The brain scan showed increased activity in the right frontal lobe. The right brachial arteriogram showed an anteriovenous malformation in the same region.

*Case No. 4:* DK was a 69-year-old woman admitted with the history of gradually declining vision in the left eye. This had been occurring over a years' period. There were no other complaints.

On examination she was noted to have a normal mental status. Gait, power and coordination was normal. Visual acuity was 20/200 in the left eye and 20/20 in the right. Visual field examination showed that the patient had a left nasal field defect to movement, a left central

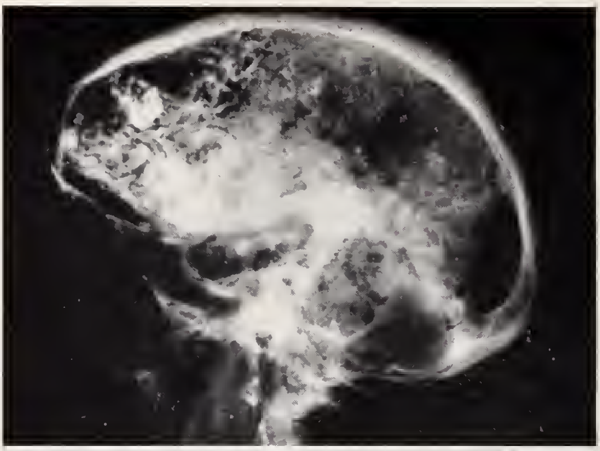

FIG. 3. Arterial phase of a cerebral angiogram showing a large vascular malformation in the second patient. The malformation occupies most of the anterior portion of the left cerebral hemisphere.

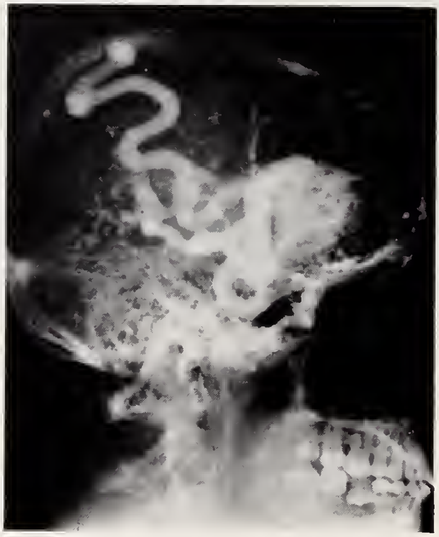

FIG. 4. Arterial phase of cerebral angiogram in third patient outlining a large frontal vascular malformation with a large draining vein from malformation to the saggital sinus.

scotoma, and a bitemporal achromatopsia to red target. Skull x-rays showed the sella turcica to be enlarged. The EEG was normal. The brain scan showed increased activity in the left inferior frontal area. Endocrine studies indicated normal pituitary function. A left carotid arteriogram showed a large left frontal arteriovenous malformation. Pneumoencephalogram showed that there was intrusion of these vessels into the third ventricle.

## Discussion

Homonymous hemianopia can occur with a lesion involving the optic tract, optic radiation and occipital lobe. Three of the patients presented had some

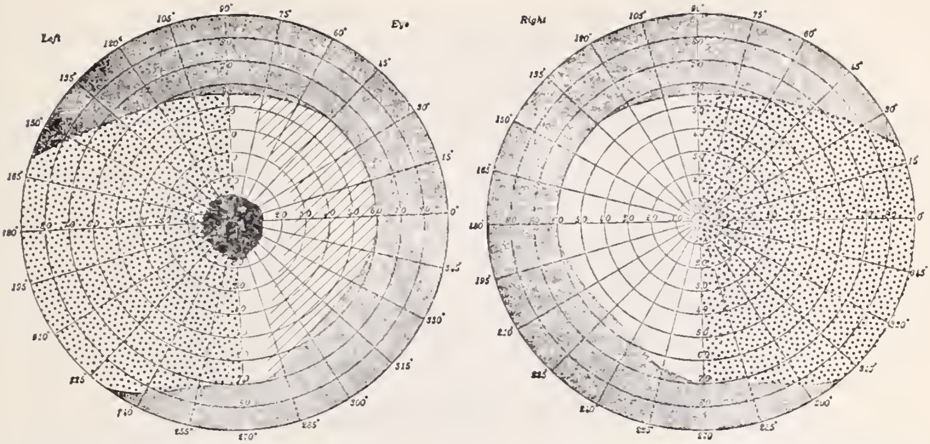

FIG. 5. Visual fields in fourth patient with a bitemporal field defect, left central scatoma, and a nasal defect to color on the left.

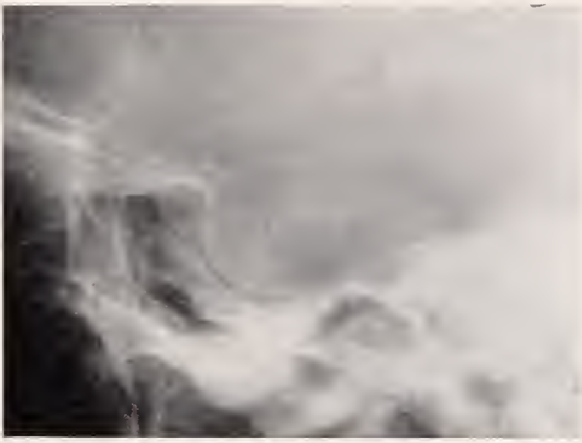

FIG. 6. Skull x-rays of the fourth patient demonstrating an enlarged sella turcica.

form of homonymous hemianopia. In the first two patients a portion of vascular malformation as identified by angiography was close to the optic radiation. The most plausible explanation is that the vascular malformation was compressing the optic radiation. However, in one of the two (LZ) there were also some large draining veins which extended as far back as the occipital lobe so that the field defect may have resulted from occipital lobe involvement.

Paterson and McKissock presented four cases of visual field involvement in a group of 110 patients with vascular malformations (5). All four had homonymous hemianopia. In two of these patients the field defect occurred suddenly. At the time of surgery both patients were found to have a hematoma in the area of the malformation. In our two patients it is difficult to ascribe the field defect to such a hematoma since neither patient had a history suggestive of an

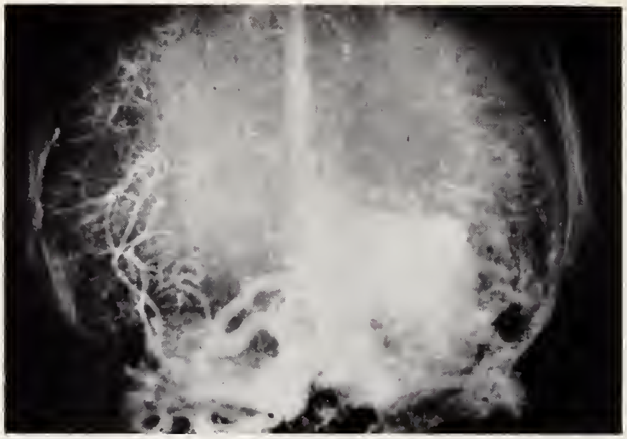

FIG. 7. Arterial phase of cerebral angiogram in fourth patient illustrating a frontal malformation with a portion of the malformation in the area of the third ventricle.

acute onset. However, in one of the patients (PL) there was a history suggestive of a bleeding episode.

In the third patient it is unlikely that the field defect arose from involvement of the optic radiation or occipital lobe, since in this patient the vascular malformation was in the frontal lobe. However, lesions of the optic tract may also cause homonymous field defects and a portion of the malformation was in the area of the optic tract.

The fourth patient did not present with a homonymous hemianopia, but rather with a temporal defect bilaterally, as well as a nasal and central defect in the left eye. This type of a field defect indicates involvement of the optic chiasm and left optic nerve. The field defects together with the sellar enlargement on the skull films suggested that the patient had a lesion of the pituitary gland. However, there was no other clinical or laboratory evidence of a pituitary lesion: angiography and pneumoencephalography had shown no evidence of same. Angiography did show a vascular malformation, a portion of which extended down into the area of the third ventricle. We can speculate that the malformation caused pressure downward on to the chiasm, causing dysfunction of the chiasm and optic nerve, as well as enlargement of the sella.

It is of interest that the patient did not show symptoms of her vascular malformation until she was 67 years of age. This raises the possibility that the effect of the malformation was not that of pressure but rather secondary to shunting away of blood from the chiasm by the malformation. The shunting became more apparent with aging when collateral circulation became impaired, also due to age.

## Summary

We have presented four cases of vascular malformation with visual field involvement. Three had homonymous hemianopia, the fourth a bitemporal defect with a left nasal and central defect. Various lesions in the optic chiasm, tract and radiation have been considered to explain the visual field defects noted.

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# RADIOLOGIC NOTES

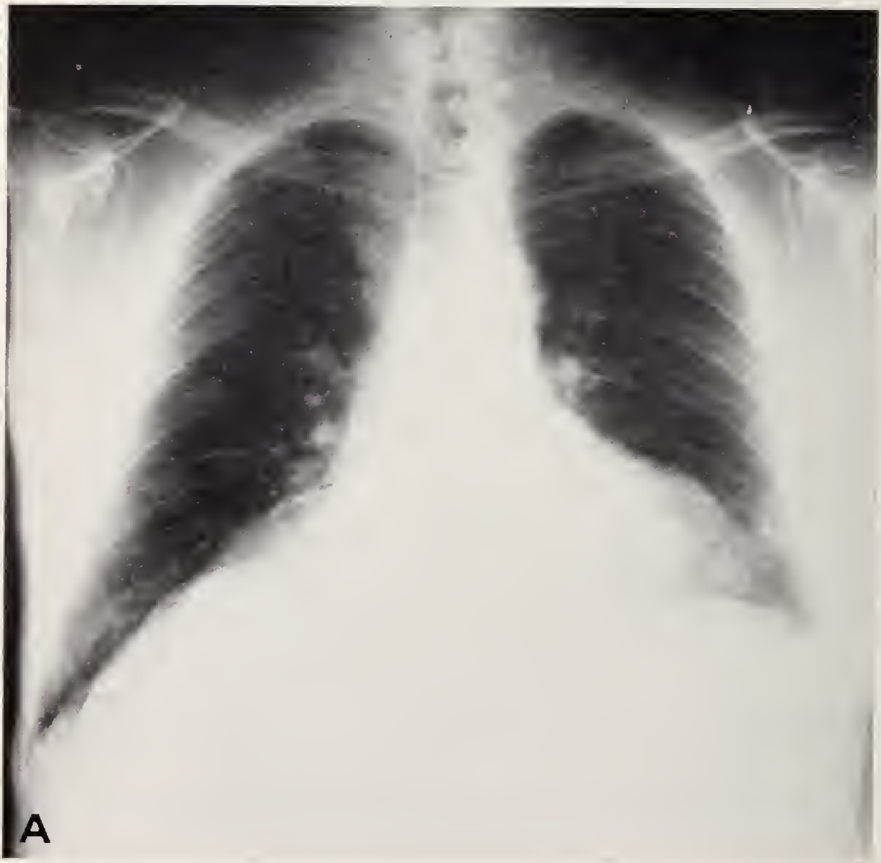
CLAUDE BLOCH, M.D. AND HARVEY M. PECK, M.D., COEDITORS

## CASE NO. 369*

*Submitted by*

MYRON MELAMED, M.D.

A 47-year-old man was admitted to the hospital with weakness of the arms and legs, and a clinical diagnosis of amyotrophic lateral sclerosis was made. Radiologic examination of the chest in the postero-anterior projection (Fig. 1 A) revealed a homogeneous density overlying the medial aspect of the right leaf of



Case 369, Fig. 1A: Postero-anterior view of the chest reveals a homogeneous density overlying the medial aspect of the right leaf of the diaphragm, oblitering the right cardiophrenic angle.

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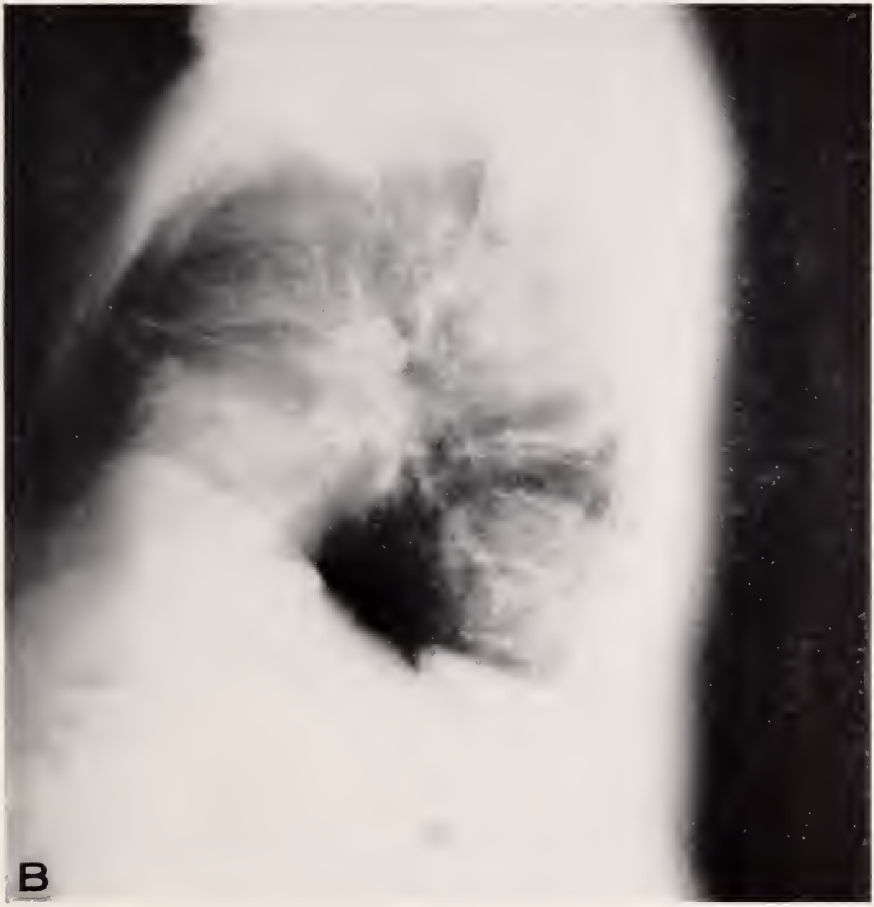
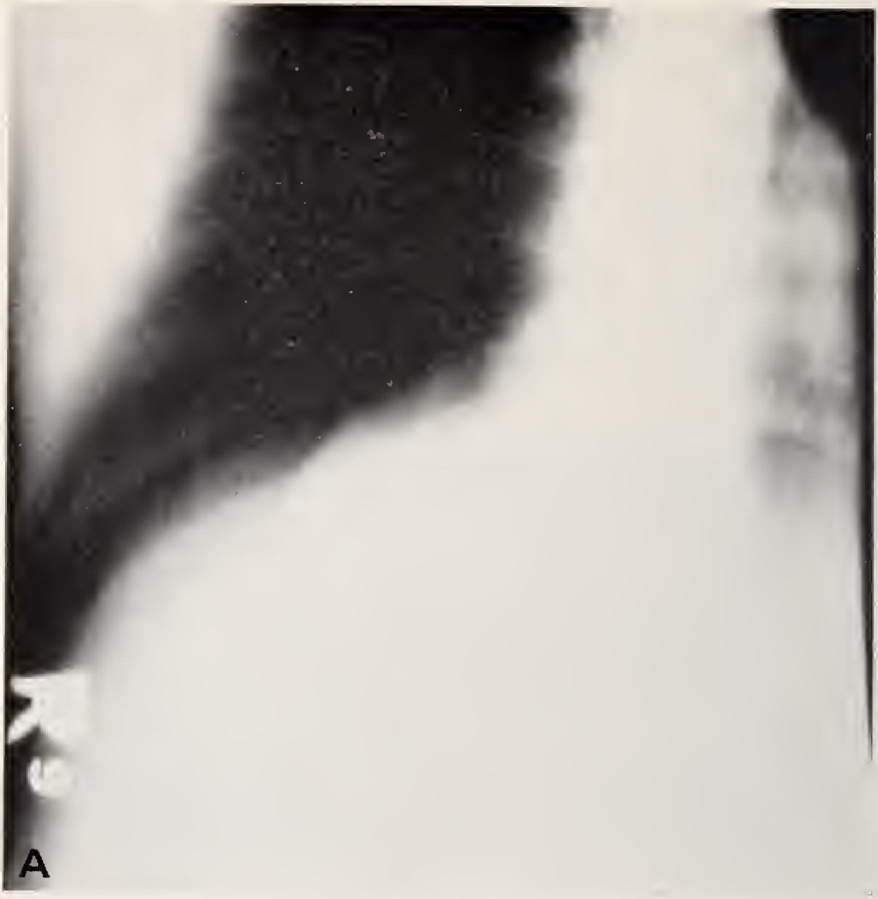
the diaphragm, obliterating the cardiophrenic angle. In the lateral projection (Fig. 1 B), the mass was noted to be situated posteriorly, overlying the vertebral bodies. Its anterior and superior borders were sharply defined, whereas its inferior margin was not seen. No lucencies, calcifications, or fluid levels were noted within the mass. Laminogram in the frontal projection (Fig. 2 A) at a posterior level confirmed the sharp outlines of the mass superiorly and laterally. It was independent of the medial portion of the diaphragm. Lateral laminogram (Fig. 2 B) demonstrated the superior and anterior borders of the mass to better advantage; inferiorly, it was broad-based, stemming from the posterior-inferior-most region of the chest. Intravenous pyelogram revealed the right kidney to be normal in position (Fig. 3). Gastrointestinal series revealed no evidence of herniation (Fig. 4).

Notwithstanding the poor prognosis of the patient, exploratory surgery was



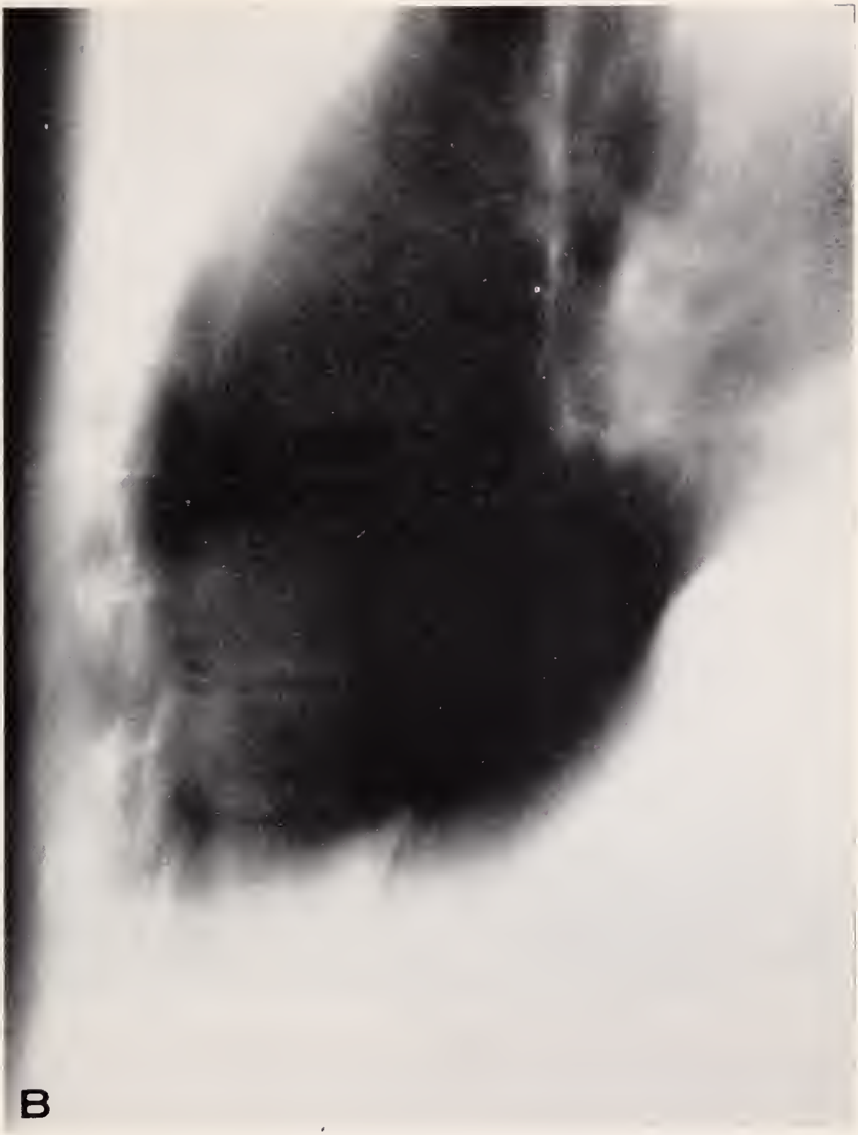
Case 369, Fig. 1B: Lateral view of the chest shows the mass to be situated posteriorly overlying the vertebral bodies. Its anterior and superior borders are sharply defined, whereas its inferior margin is not seen.



Case 369, Fig. 2A: Antero-posterior laminogram taken at a posterior level confirms the sharp outlines of the mass superiorly and laterally. No unusual calcifications or gas collections are noted within the mass.

performed in order to rule out malignancy and any relationship to the neuromuscular disorder. The possibilities of neurogenic tumor and foramen of Bochdalek hernia were considered. A right thoracotomy was performed. The lung was normal to inspection and palpation. Posteriorly, the gutter behind the diaphragm was completely occupied by a well-encapsulated mass consistent with fat. The mass was covered with a serous membrane and had a pedicle at the absolute posterior-inferior portion of the retrodiaphragmatic sulcus. It became evident that a five- to six-centimeter defect was present in the diaphragm through which extruded the retroperitoenal perirenal fat. The right kidney was below the diaphragm. The herniated fat and enveloping sac were removed and the diaphragmatic defect was repaired.

Pathology specimen consisted of a ten-centimeter mass of fibro-fatty tissue.

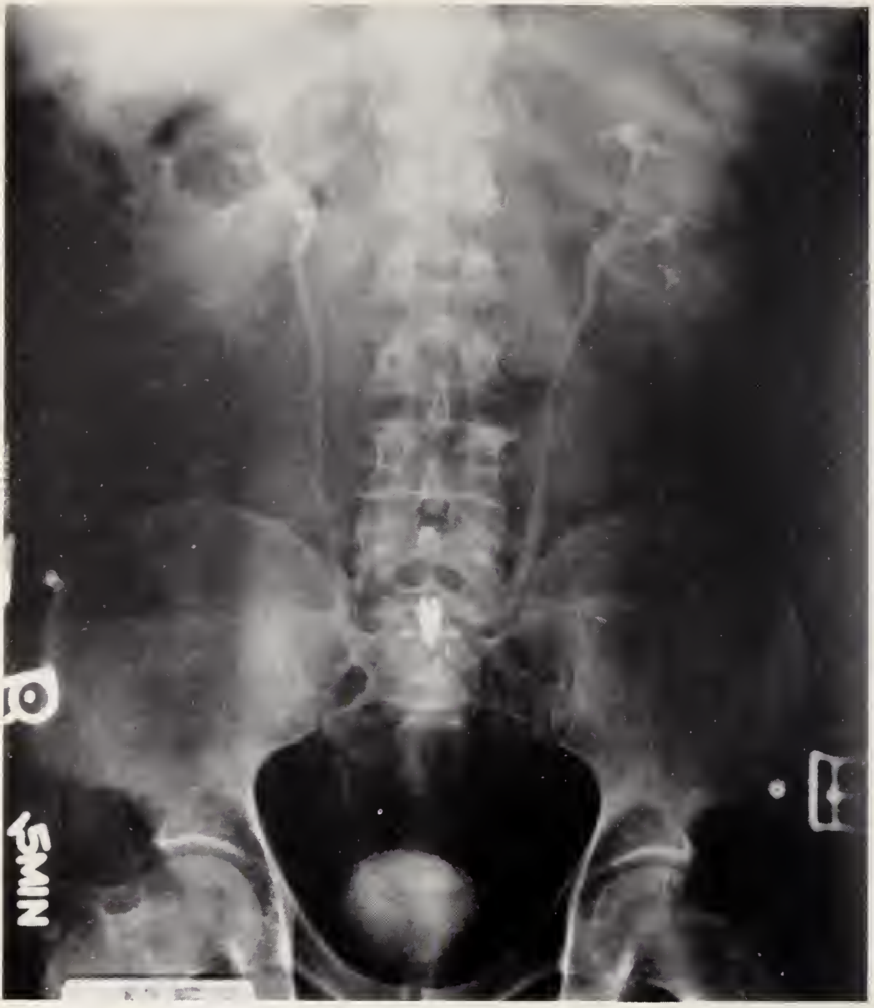


Case 369, Fig. 2B: Lateral laminogram demonstrates the superior and anterior borders of the mass to better advantage; inferiorly, it is broad-based, stemming from the posterior-inferior-most region of the chest.

Microscopic sections revealed well-encapsulated lobules of benign adipose tissue. There was no evidence of inflammation or malignancy.

## Discussion

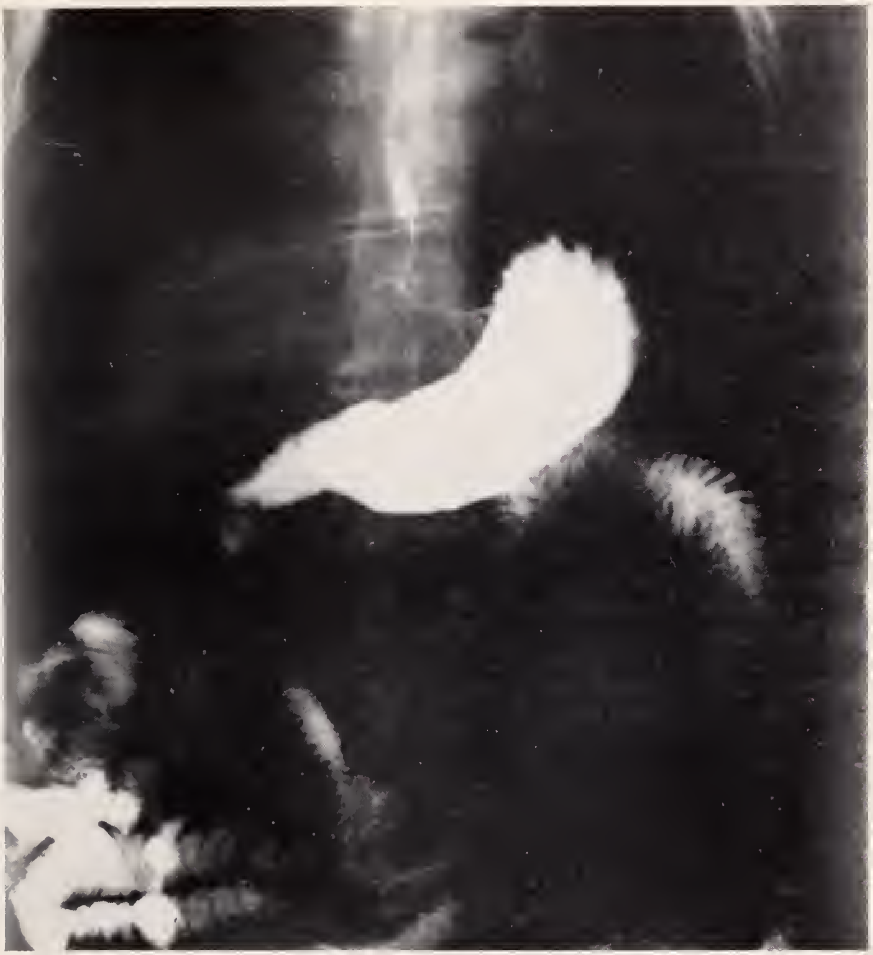
Pleuroperitoneal hiatus hernia, or hernia through the foramen of Bochdalek, represents a failure of fusion of pleuroperitoneal membrane with the septum



Case 369, Fig. 3: Intravenous pyelogram reveals the right kidney to be within the range of normal in position. Incidentally noted is a small amount of residual contrast medium in the spinal canal relating to previous myelography.

transversum. Usually there is no hernia sac and there is direct communication between abdominal and thoracic cavities. This condition is generally found in infants, infrequently in adults. Of four hundred and thirty cases of diaphragmatic hernia reported by Harrington (9), only nine represented Bochdalek hernias—all in infants. Pleuroperitoneal hernias are relatively rare in adults. Forty-seven cases have been reported in the literature to date (1–9). In only one other instance was the hernia content listed as perirenal fat similar to the case herein presented (1).

Symptoms of Bochdalek hernias in the adult can vary from the negligible to



Case 369, Fig. 4: Gastrointestinal series reveals no herniations.

those of an apparent catastrophic event simulating coronary thrombosis, dissecting aneurysm of the aorta, perforated viscus, or tension pneumothorax. Serious symptoms can relate to strangulation of a viscus and/or acute tension (6–8). In such cases, appropriate diagnosis and therapy become imperative. In most instances, symptoms are moderate and intermittent, reflecting transient herniation of an organ.

Radiographic differential diagnosis can be divided into two main categories, depending on whether the herniated organ is solid- or gas-containing. In the former instance, posterior mediastinal masses such as neurogenic tumors represent the major consideration. If the herniated organ is gas-containing, chest radiographs may simulate pneumothorax, giant lung bullae, obstructive emphysema, or eventration. The position of the diaphragm may be difficult to establish. Generally, the herniated organs are stomach, small bowel and/or colon. However, herniation of liver, spleen, pancreas, and omentum have been described, as well as retroperitoneal fat. High ectopia of the kidney is not included in this reported series.

*Case Report:* HERNIATION OF RETROPERITONEAL FAT THROUGH THE FORAMEN OF BOCHDALEK IN AN ADULT.

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## CASE NO. 370*

*Submitted by*

SHELDON H. CALEM, M.D. AND RHONA J. KELLER, M.D.

A 41-year-old man had several months' history of vague abdominal pains. Rectal examination revealed a pelvic mass. Sigmoidoscopy was negative. A diagnosis of appendiceal abscess had been made and conservative management was selected. One year later, the patient passed bright red blood per rectum.

Barium enema revealed a grapefruit-sized pelvic mass elevating the sigmoid (Fig. 1 A). In the mid-sigmoid region, there was a large elliptical collection of barium (between arrows) with slightly nodular inferior margins. In the oblique view (Fig. 1 B), the ulceration stood out clearly (between arrows) and was noted to straddle the superior surface of the pelvic mass. There was no evidence of colonic obstruction or proximal dilatation. The adjoining portions of the sigmoid appeared to be normally distensible. In the lateral projection, (Fig. 1 C), the distal sigmoid was noted to be displaced posteriorly by the pelvic mass which caused a smooth narrowing of the bowel lumen (between

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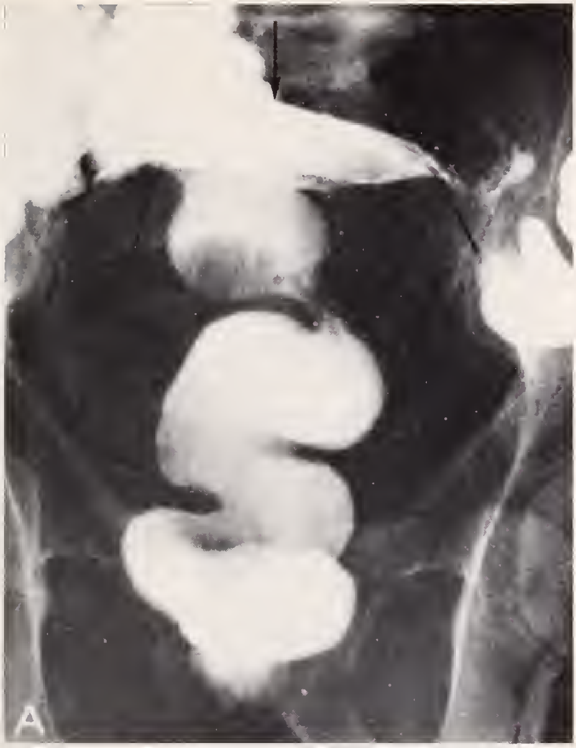


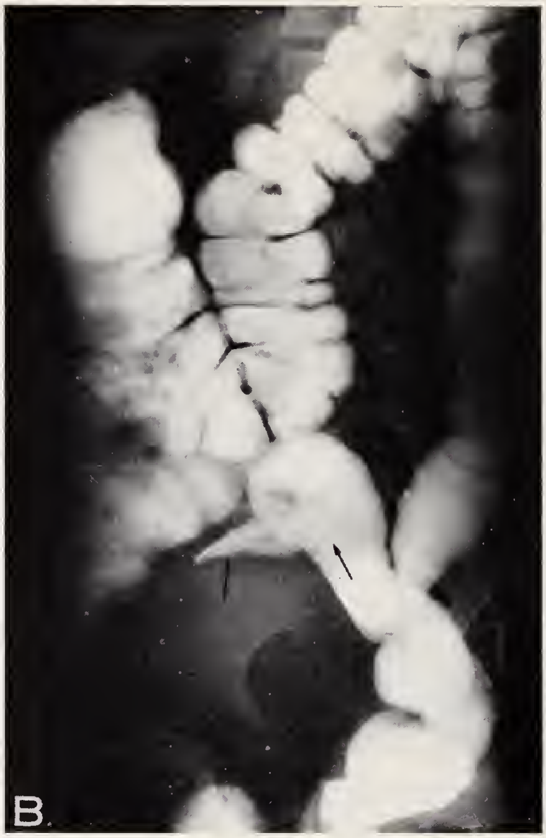
Case 370, Fig. 1A: Antero-posterior view of the barium-filled sigmoid reveals a grapefruit-sized pelvic mass elevating the sigmoid. There is a large elliptical collection of barium (between arrows), with slightly nodular inferior margins. This represents a large ulceration.

arrows). Air contrast study (Fig. 1 D) showed the ulcer to be filled with barium (along arrows), whereas the adjoining portion of the circumference was distended by air. This indicated that only a portion of the sigmoid circumference was ulcerated and fixed to the mass. The nodularity of the inferior margin of the ulceration was clearly noted in this view.

The patient was explored and a firm, nodular tumor, 16 centimeters in diameter, was found adherent to the sigmoid, fixing and distorting this segment of bowel. The sigmoid contained an ulceration 6 centimeters in length. The entire area was resected en masse, and an end-to-end sigmoid anastomosis was performed. The patient made an uneventful recovery. The tumor, on gross examination of the cut surface, had a whitish-grey color. Focal areas of cystic degeneration and necrosis were present (Fig. 2).

## Discussion

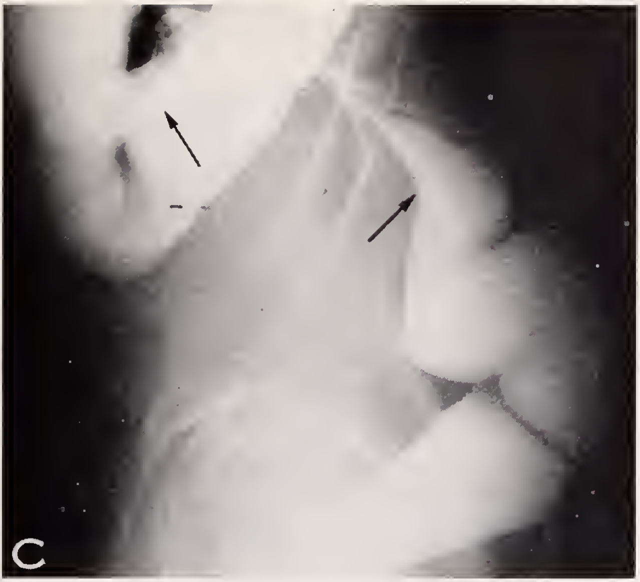
The radiographic appearance of a myosarcoma is largely determined by its position in the bowel wall. The neoplasm may be intramural, submucosal and



Case 370, Fig. 1B: Left anterior oblique view of the barium filled colon demonstrates the ulcer clearly (between arrows); the ulcer seems to straddle the superior portion of the pelvic mass.

intraluminal, subserosal, or of the dumbbell type (a combination of the first three) (1). A constrictive type is also described which is rare and which may narrow the bowel lumen. Intramural tumors are rarely identified because they are usually small, confined to the wall, and asymptomatic.

Radiographically, the submucosal tumor presents within the lumen as a well-circumscribed exquisitely smooth, sometimes lobulated eccentric defect, broad-based and covered by a stretched but intact mucosa. An acute angle is seen at the junction of the defect and the normal bowel wall. There is usually no rigidity of the wall and proximal obstruction is rare. The tumor may ulcerate, and commonly does so in the gastrointestinal tract distal to the esophagus. Ulcers in small benign tumors may be smooth, in larger masses, they tend to be irregular. In large sarcomatous lesions, ulcers tend to be very extensive with multiple fistulae extending into the substance of the neoplasm. Rapid tumor

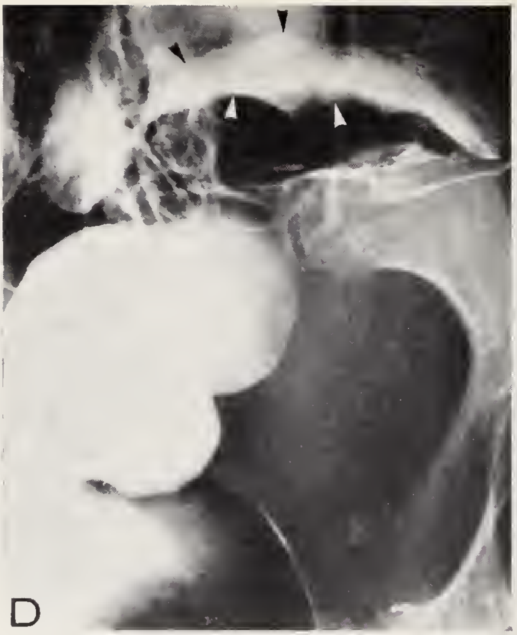


Case 370, Fig. 1C: In the lateral projection, the sigmoid is noted to be displaced posteriorly by the pelvic mass, which also causes a smooth narrowing of its lumen (along arrows).

growth, large, ragged ulceration, and multiple long fistulae favor malignant change. The subserosal tumor presents as a mass outside the wall with edges that taper and form an obtuse angle with the bowel wall. Adjacent structures or loops of bowel may be displaced by the tumor mass, giving some indication as to its size. Ulceration, if present, extends into the bulk of the tumor, and tracks of barium may be seen extending from the lumen into the mass. These tumors remain asymptomatic for long periods of time and are diagnosed late. The combined dumbbell-type lesion demonstrates the characteristics of both the submucosal and subserosal types. Constrictive lesions may be found with the tumor growing circumferentially, producing a constriction with or without an intact overlying mucosa (2–4).

Metastatic lesions, notably from the stomach, ovary, or local extensions from pelvic tumors such as prostatic carcinoma, may present a similar appearance radiographically. However, these lesions are usually multiple and are more likely to produce obstruction. Giant-cell tumors of the sacrum may present a similar appearance. Mucosal tumors such as adenomas or polyps are lobulated and may or may not exhibit a pedicle. Grossly irregular margins favor carcinoma (2, 4).

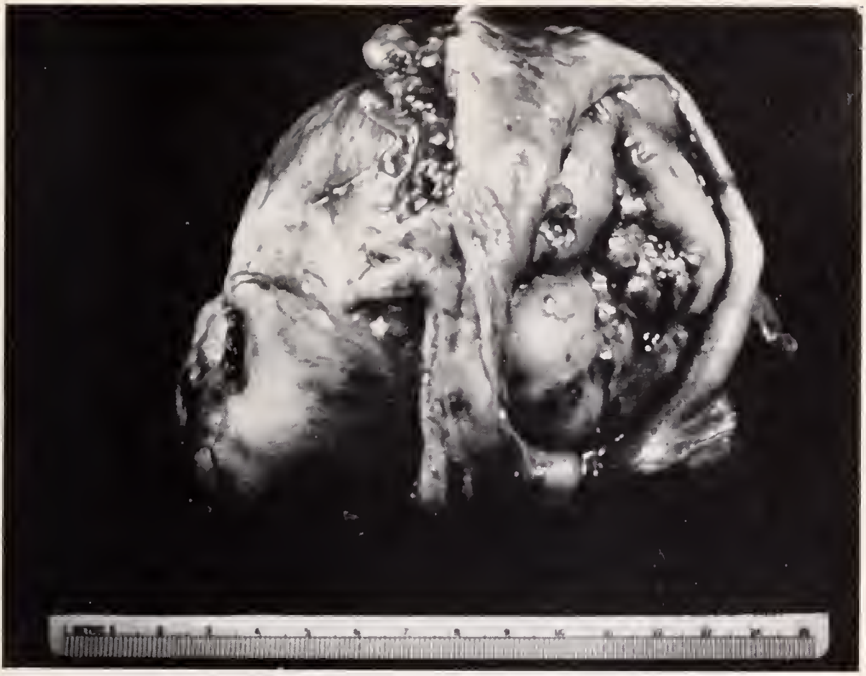
The treatment of this disorder is primarily surgical with wide local excision. Radiotherapy and chemotherapy have been used with little success (5, 6). Leiomyosarcomas have a great tendency to metastasize to the liver, spread to



Case 370, Fig. 1D: Air contrast study of the sigmoid in the right anterior oblique projection reveals the ulcer to be filled with barium (along arrows), whereas the adjoining portion of the circumference is distended by air. The large pelvic mass is well seen inferiorly.

the peritoneum, involve the lungs, or occasionally present with lymph node metastasis. Survival is usually from 6 to 18 months (7). In general, leiomyosarcomas of the rectum have a worse prognosis than leiomyosarcomas of other parts of the colon owing to absence of the anatomic barrier of the peritoneum, which temporarily prevents extension of the tumor into contiguous tissues.

Smooth muscle tumors comprise approximately one percent of all tumors of the gastrointestinal tract (8). There is no sex predilection. The peak incidence is in the 40 to 60 age group. The average duration of symptoms is approximately one to six months (9). The cardinal presenting signs are bleeding, obstruction and palpable mass (7, 10–12). These tumors involve the stomach, small intestine, esophagus, and colon in descending order of frequency. They are twice as common in the rectum as in any part of the large bowel (10, 13). The ratio of leiomyosarcoma to leiomyoma is greatest in colonic tumors. This fact may be reflected in the relatively poor prognosis of smooth muscle tumors of the colon. The sarcomatous lesions are usually over 5 cm in size and, the more malignant the neoplasm, the more likely it is to undergo central necrosis and excavation. Ulcers are almost never seen in tumors of the esophagus but commonly occur in the more distal portions of the bowel (8).



Case 370, Fig. 2: Surgical specimen showing the mass opened from above. The central elliptical ulceration is clearly noted with nodularity of its base.

### Acknowledgments

The editors wish to thank Dr. Leon Ginzburg and Dr. A. Daniel Hauser for permission to publish this case.

*Case Report:* LEIOMYOSARCOMA OF THE SIGMOID COLON.

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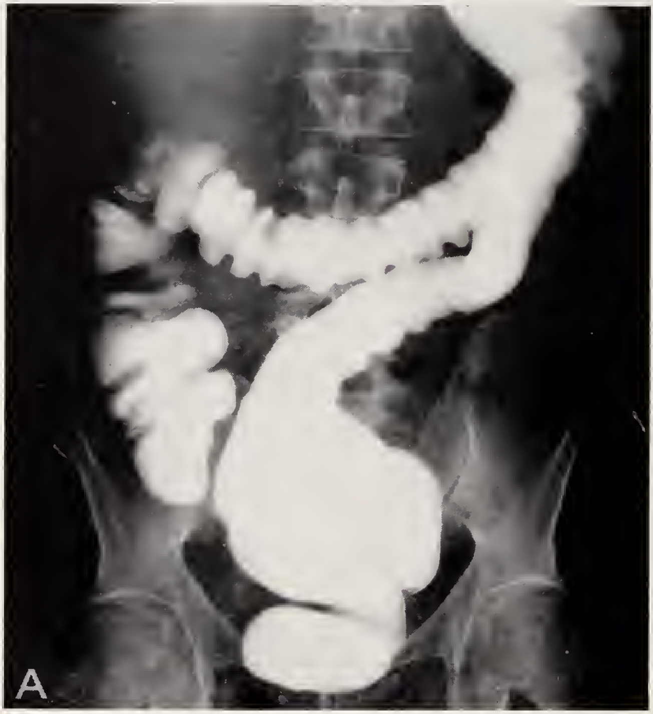
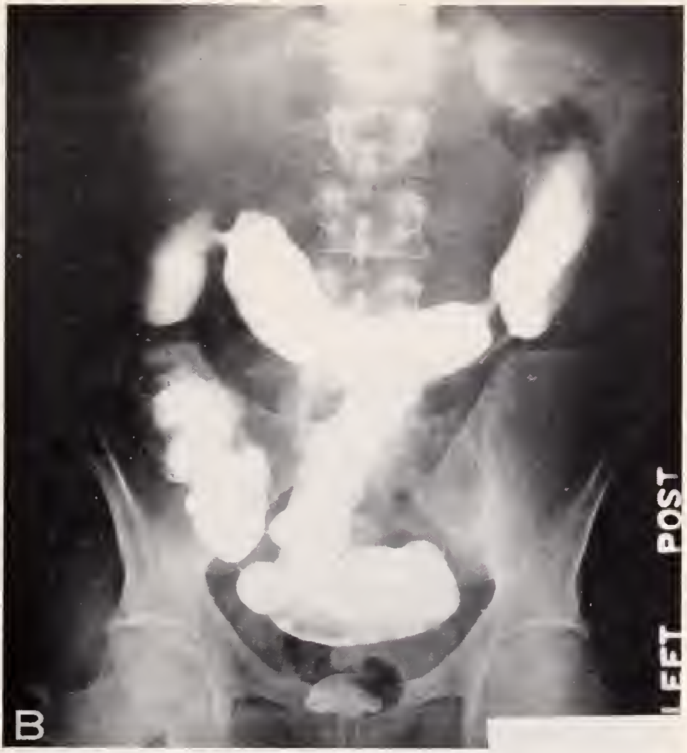
## CASE NO. 371

This 42-year-old man presented with the chief complaint of abdominal cramps and diarrhea. He first noted intermittent attacks of diarrhea eight years previously and had been treated with nonspecific means. An exacerbation occurred after a trip to Yugoslavia and the diarrhea and tennesmus became intense and unrelieved by antispasmodics. Physical examination revealed hyperactive bowel sounds, but the abdomen was soft and no masses were felt. Barium enema examination revealed slight generalized lack of distensibility of the



Case 371, Fig. 1A: The barium-filled colon reveals a slight general lack of distensibility with flattening and irregularity of the haustral pattern, especially in the proximal two-thirds of the large intestine. No discrete ulcerations are identified, but the mucosa in the ascending and transverse colon appears thickened and distorted.



Case 371, Fig. 1B: On the postevacuation study, the haustrations appear completely lacking throughout the large intestine. There appears to be considerable edema of the walls of the transverse colon, and the visualized mucosa in the descending colon appears spiculated, suggesting superficial erosions. After specific medical therapy for the amebiasis, the patient made a complete clinical recovery and repeat barium enema showed a normal colon.

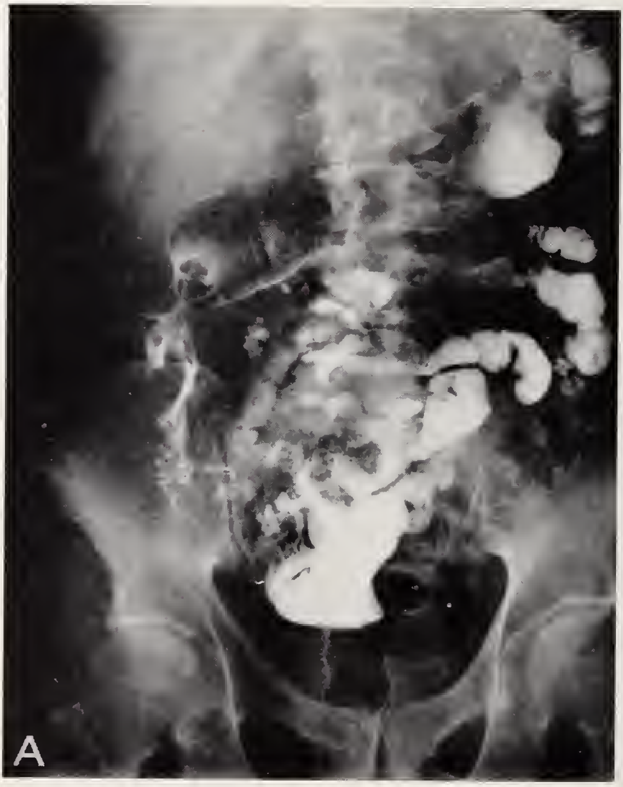
colon, with flattening and irregularity of the haustral pattern, most marked in the proximal two-thirds of the colon (Fig. 1 A). The mucosa appeared edematous in the transverse and descending colon; this was best demonstrated on the postevacuation radiograph (Fig. 1 B). Proctoscopic examination revealed large internal and external hemorrhoids. Mild erythema of the rectal and sigmoid mucosa was noted without discrete ulcerations. No anal or perirectal fistulae were noted. The patient was placed on chloroquine therapy and made a dramatic symptomatic recovery. Repeat barium enema after treatment was normal.

### Acknowledgments

The editors wish to thank Dr. Sidney Greenberg, Dr. Robert Turell and Dr. Charles M. Newman for permission to publish this case.

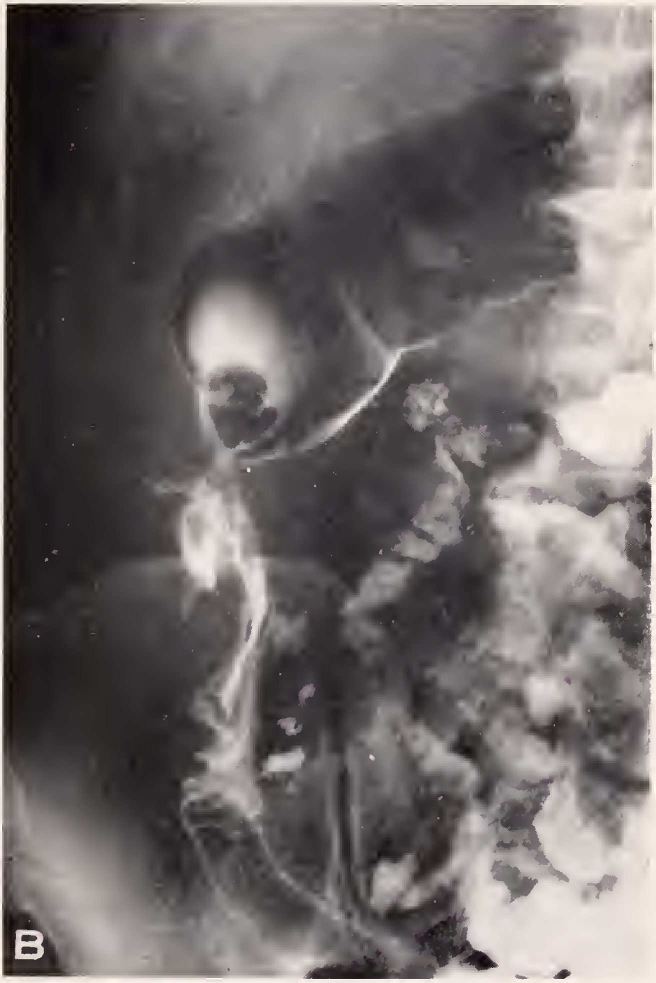
## CASE No. 372

This 73-year-old man had a 20-year history of amebic dysentery, having lived extensive periods of time in South America. He now presented with a four-months' exacerbation of symptoms with mucous diarrhea, tennesmus, weight loss, and abdominal pains. Barium enema revealed marked shortening of the ascending colon (Figs. 1 A & 1 B) and an eccentric narrowing associated with an ulcerated mass in the distal transverse colon (Figs. 1 B & 1 C). The transverse colon was noted to lack normal haustrations. The terminal ileum appeared normal. No fistulae were seen. The patient was explored in order to rule out the

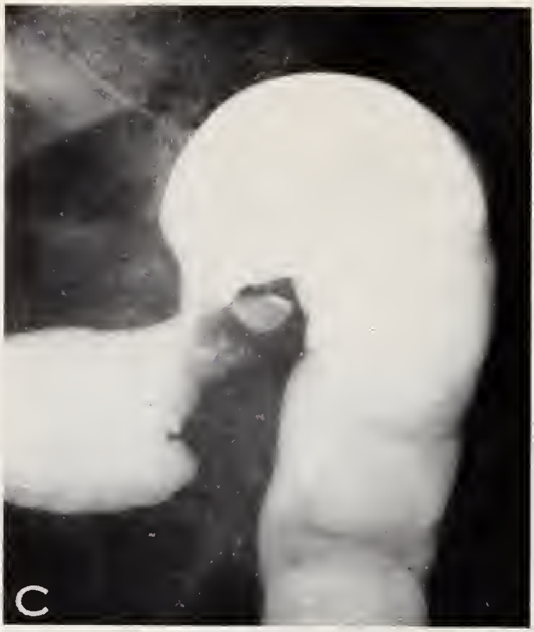


Case 372, Fig. 1A. Barium enema reveals a marked distortion and shortening of the ascending colon and an eccentric narrowing of the distal transverse colon. On the right side of the colon, there is a concentric narrowing and shortening of the lumen and the mucosa is effaced and distorted, with a deep, cleft-like projection laterally, suggesting an ulceration. The transverse colon is ahaustral. Within the proximal limb of the splenic flexure there is an ulcerating lesion causing an eccentric narrowing of the bowel. An irregular, shallow ulceration is noted within the mass. Incidentally noted is sigmoid diverticulosis. There is no evidence of proximal bowel dilatation or fistula formation.

possibility of a superimposed malignancy in the large intestine. A subtotal colectomy was performed. At operation, the ascending colon was markedly shortened and the cecum itself was absent. In the right lower quadrant, there was a fist-sized inflammatory mass surrounded by adhesions. Another fibrotic mass was present on the left side of the colon, which did not have the gross appearance or consistency of a malignancy. The opened colon, in the region of the splenic flexure, contained an irregular, sharply demarcated ulceration surrounded by normal mucosa, situated near the apex of the mass itself. A second smaller independent ulceration was noted adjacent to the larger ulcer.



Case 372, Fig. 1B. Spot film of the right side of the colon confirms the presence of the markedly narrowed and shortened ulcerated ascending colon.



Case 372, Fig. 1C. Spot film of the splenic flexure demonstrates details of the irregular ulcerations within the mass affecting the distal portion of the transverse colon. The ulcerations are noted to be multiple and irregular in outline. No overhanging edges are seen and the bowel lumen is noted to be eccentric.

### Acknowledgments

The editors wish to thank Dr. Carlos Diaz, Department of Surgery, V.A. Hospital in Brooklyn, N.Y., and Dr. Jack Forman, Department of Radiology, Downstate Medical Center, State University of New York, for permission to publish this case.

## Discussion

### Cases 371 and 372

The overall incidence of amebiasis in the United States is about five percent. Following ingestion, the trophozoite (Entamoeba histolytica) penetrates the colonic mucosa and lodges in the submucosal glands. The parasite can, however, inhabit the host without causing a tissue reaction and thus the patient is an asymptomatic carrier. Once it is localized within the mucosa, varying degrees of tissue reaction occurs with the development of ulcerations, at first punctate and sharply demarcated with overhanging edges due to undermining (1). The involvement may be diffuse, resembling ulcerative colitis (2). The ascending colon is most often involved when the disease is localized (90% of cases), and the terminal ileum is almost always spared.

In early cases, the colon simply shows irritability and slight lack of distensibility with some mucosal edema and irregularity of haustration (3). When the right side of the colon is primarily affected and the rectum is spared, amebic colitis mimics granulomatous colitis (Crohn's disease of the colon). In chronic cases, an ameboma may form, resembling colonic neoplasm (4). These amebomas represent granulomas with mucosal ulcerations and formation of an inflammatory mass lesion. Toxic dilatation of the colon has been reported as a complication in the fulminating case of amebic colitis (5). In a few reported cases, perforation may occur, causing peritonitis, or, if walled off, sinus tracts or internal fistulae may result (6).

In the differential diagnosis of early and diffuse cases, amebic colitis resembles ulcerating or granulomatous colitis. The terminal ileum is usually spared, being affected in only three percent of 200 autopsied cases (7), as opposed to cases of granulomatous colitis. Slight residual pliability of the colonic walls, multiple lesions and lack of obstruction favor the diagnosis of chronic amebiasis with ameboma formation versus colonic malignancy. In almost all cases of amebic colitis, whether acute or chronic, including case no. 371 presented here, there is marked improvement and usually complete radiologic reversibility after proper antiamebic therapy. It is usually desirable not to intervene surgically in cases of amebic colitis, since the inflamed bowel walls heal poorly and there is up to 50% mortality (8).

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